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CHEMICAL INVESTIGATION IN CONNECTION WITH LEPROSY INQUIRY.

PART II.

BY

DR. SUDHAMOY GHOSH, D.SC., F.R.S. (Edin.), M.SC. (Cal.), F.C.S. (Lond.).

(Chemical Laboratory, Medical College, Calcutta.)

[Received for publication, July 7, 1920.]

IN the following communication an account is given of the results which have been obtained during the chemical investigation of chaulmoogra oil and some closely related oils with the object of testing their efficacy in the treatment of leprosy.

I. *Oil from Taraktogenos Kurzii.*—(Chaulmoogra Oil.)

The chemistry of this oil was carefully investigated by Power and Barrowcliff (*Journ. Chem. Soc. Trans.*, 1905, 896). An attempt by the author to separate the oil by fractional distillation under diminished pressure was unsuccessful as it decomposed. The free fatty acids also decomposed when distilled at 40 mm., the lowest pressure obtainable.

The oil used had the following constants :—

	By Butyro-refractometer.	By Abbe-refractometer.
Oil extracted with light petroleum ..	1·4729/40°	1·4747/31·4°
Oil extracted with ether	1·4723/40°	1·4733/31·4°

The neutralisation value of the total acids obtained from the oil was found to be 142·6 milligrams NaOH.

Samples of the oil obtained from the seeds both by extraction with light petroleum and by pressing were hydrolysed and the fatty acids carefully fractionated by crystallisation from alcohol. It was found that the fraction melting at about 50° was the most suitable for use.

This seems to point to the fact that hydnocarpic acid and some of its homologues are really the 'active principle' in chaulmoogra oil. Chaulmoogric acid itself cannot be used owing to the sparing solubility of its salts. An attempt was made to get over this difficulty by mixing pure chaulmoogric acid with some of the lower melting fatty acids but the sodium salt of chaulmoogric acid separated on standing for a day.

II. *Oil from Hydnocarpus Wightiana.**

The seeds from this tree, which is indigenous to the West of the Indian Peninsula, yield another variety of chaulmoogra oil. The oil examined was supplied by Messrs. Smith, Stanistreet & Co. The specific gravity at 22° was 0·9548 and it gave on hydrolysis a mixture of fatty acids melting at about 40°. These acids were fractionally crystallised from alcohol when the most sparingly soluble fraction was found to consist mainly of chaulmoogric acid (about 7·3 per cent), the second fraction (about 10 per cent) being mainly hydnocarpic acid. The sodium salt of the second fraction was found to be most efficacious and convenient for use in the treatment of leprosy.

III. *Oil from Hydnocarpus Venenata.*

This tree, which is known as the small chaulmoogra or small kalawathi, occurs in Burma and also in Madras and Ceylon.

The seeds when cold and hot pressed gave the following proportions of oil—Cold : Hot = 2·02 : 1. A mixture of the cold and hot pressed oils had the following constants :—M. P. about 20°, $D\frac{25°}{25°}$ 0·9536, iodine value 99·69, neutralisation value of total fatty acids 203·4 milligrams of KOH, M. P. of total fatty acids 41—44°. In chloroform solution the oil was dextro-rotatory.

The fatty acids were fractionally crystallised from alcohol when the least soluble fraction (about 9·4 per cent) consisted of almost pure chaulmoogric acid, whilst the second fraction (about 8·7 per cent) was found to be mainly hydnocarpic acid. The sodium salt of the second fraction was found to be most suitable for injection.

An attempt was made to use the *ethyl ester* of the total fatty acids but its use was discontinued.

From the ethereal extract of the hydrolysed oil phytosterol was isolated.

---

* C. J. Lewkowitsch.—*Oils, Fats and Waxes*, 5th Ed., Vol. II, pp. 493–495.

IV. *Oil from Asteriostigma Macrocarpa.*

This tree is known as the large chaulmoogra or big kalawathi, and occurs in Burma and Madras.

The seeds when cold and hot pressed gave the following proportions of oil—Cold : Hot = 3·02 : 1. A mixture of the cold and hot pressed oils had the following constants :—M. P. about 20°, $D\frac{25°}{25°}$ 0·9519, iodine value 98·38, saponification value 202·7. The oil was dextro-rotatory in chloroform solution.

Fractional crystallisation of the fatty acids from alcohol yielded chaulmoogric acid (about 8·0 per cent) and hydnocarpic acid (about 11 per cent). The ethereal extract of the hydrolysed oil contained phytosterol.

V. *Oil from Hydnocarpus Alpina.**

The seeds of this tree were sent as a Nilgiri species of chaulmoogra. The seeds were much smaller than those from other varieties. The seeds when cold and hot pressed gave the following proportions of oil—Cold : Hot = 12 : 1. The oil had a deep green fluorescence but after treatment with animal charcoal, it was light yellow in colour and possessed the usual smell of chaulmoogra oil. The following constants were observed :—$D\frac{25°}{25°}$ 0·9518, $N^{D40°}$ 1·4716, $[\alpha]_D^{22°}$ + 50 (approximately), saponification value 199·9, iodine value 92·42, unsaponifiable matter 3·1 per cent, M. P. of total fatty acids 45—48°, $[\alpha]_D^{22°}$ +52·96 (c = 2·9266) (approximately), neutralisation value 210·6, iodine value 95·91.

Sufficient of the fatty acids were not obtained for a detailed examination, but the fraction obtained after crystallisation from alcohol and melting at about 50° was utilised in the treatment of leprosy.†

Some Derivatives of Chaulmoogric Acid.

In spite of its close relationship to hydnocarpic acid (it is probably a higher homologue) chaulmoogric acid has, owing to the sparing solubility of its sodium salt, not proved suitable for the treatment of leprosy. An

---

* (C. J. Lendrich, Koch and Schwarz, Zeits. G. Unters. Nahrgs-U-Genussm 1911, 22, 441. Wolff and Koldewijn. Pharm. Weekblad, 1912, 1049.)

† Attention may be directed to the fact that the oils from H. Wightiana and H. Venenata are much cheaper than the oil from T. Kurzii, whilst they contain a larger percentage of hydnocarpic acid, *i.e.*, about 10 per cent as compared with 5·5 per cent. It would appear, therefore, more economical to use the oil from H. Wightiana in place of the oil from T. Kurzii for leprosy treatment.

attempt has been made to prepare from it a series of derivatives which might be of medicinal value ; a full account of their properties will appear elsewhere. The following substances were prepared :—*chaulmoogric acid anilide* $C_{17}H_{31}CO\text{-}NH\text{-}C_6H_5$, *chaulmoogric acid phenylhydrazide* $C_{17}H_{31}CO\text{-}NH\text{-}NH\text{-}C_6H_5$, *chaulmoogric acid ureide* $C_{17}H_{31}CO\text{-}NH\text{-}CO\text{-}NH_2$, *chaulmoogric acid urethanide* $C_{17}H_{31}CO\text{-}NH\text{-}CO_2C_2H_5$, *chaulmoogric acid ozonide* $C_{18}H_{32}O_5$, *chaulmoogric acid per-ozonide* $C_{18}H_{32}O_6$. Unfortunately all these substances are insoluble in water and hence their therapeutic value could not be tested by injection. They may, however, prove of use in the treatment of surface wounds, and in the case of the ozonides the oxygen evolved on decomposition may have some effect. The ozonides of ethyl and methyl chaulmoograte, which were obtained as oils, were also prepared.

Experiments with Cod Liver Oil.

O. T. Williams (*Pharm. Journ.*, 1912, 806) showed the beneficial effects of cod liver oil on patients suffering from phthisis and stated that the fatty acids of the oil actually dissolved the waxy envelope surrounding the tubercle bacillus, and suggested the possibility of this taking place in the body. Sir Leonard Rogers made a further advance by suggesting that the pure sodium salt of the fatty acids might be a more useful agent in the treatment of tuberculosis.

Experiments were, therefore, started using pure Norwegian oil. The fatty acids were liquid at the room temperature (about 24°) and no attempt was made to isolate the different constituents. In the earlier experiments the sodium salt was highly coloured and a long series of experiments were instituted with the object of preparing this salt on a large scale in a pure condition. The method finally adopted was to hydrolyse the oil with alcoholic potassium hydroxide solution in the cold, remove the alcohol under diminished pressure, dilute with a large volume of water to separate the unsaponifiable matter, and finally extract the alkaline solution with ether. The fatty acids were then liberated by acidification, thoroughly washed and after solution in alcohol exactly neutralised with sodium hydroxide solution. This salt, to which the name *sodium morrhuate* is given, has yielded very encouraging results not only in the treatment of tuberculosis but also in leprosy.

The application of this salt in the treatment of leprosy seems to indicate that it is the *unsaturated nature* of the fatty acids present both in chaulmoogra oil and cod liver oil which cause them to be effective. This

view is supported by some very remarkable results which have been obtained by the use of the sodium salts of the fatty acids derived from soya bean oil. These acids which consist mainly of oleic and linolic acids are highly unsaturated.

These observations promise to widen considerably the field of medical research and should yield fruitful results. Further work on similar oils is in progress.

In conclusion, the author wishes to express his best thanks to Sir Leonard Rogers for his great assistance in securing materials and to Major R. Knowles, I.M.S., for the keen interest he has taken in the work and for the help he has rendered. The author also wishes to thank the Indian Medical Research Fund Association for financial assistance.

THE VACCINE INSTITUTE, BELGAUM.

A RECORD OF ITS HISTORY AND WORK.

BY

R. W. FISHER, Esq., M.B., D.P.H.,

Director of the Institute.

[Received for publication, May 5, 1920.]

History of Vaccination in Bombay Presidency up to the opening of the new Institute.

The necessity for the introduction of animal vaccine lymph, to replace arm-to-arm methods of vaccination in Bombay Presidency, was brought to the notice of the Government of Bombay by the Government of India in the year 1889.

The Sanitary Commissioner for the Government of Bombay, after consultation with his Deputies, submitted certain proposals and reported to Government in 1903, that certain experimental trials with lanolinated preserved lymph, carried out by Captain* F. H. G. Hutchinson, I.M.S., Deputy Sanitary Commissioner, Southern Registration District, in the districts under his charge, had given encouraging results.

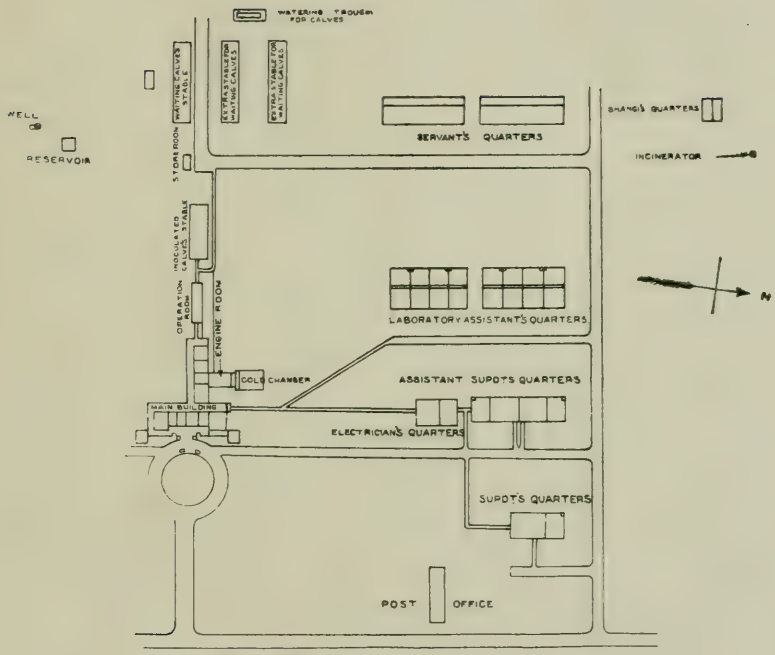
A scheme was accordingly prepared, and approved by Government, for preparing preserved lymph for use in the Southern Registration District and lanolinated lymph was supplied to the vaccinators in 1904-05.

Hutchinson had taken over the duties of Deputy Sanitary Commissioner, on 21st July, 1902, and continued to experiment with lanolinated vaccine.

At this time there was a controversy going on, amongst those engaged in vaccination work in India, regarding the rival claims of lanolinated, as against glycerinated, lymph. Lieut.-Col. W. G. King,[1] C.I.E., I.M.S., (now retired), who was then in charge of the King Institute, Guindy, Madras, was a strong advocate of lanolinated lymph, while

* Now Lieut.-Col. F. H. G. Hutchinson, I.M.S., Sanitary Commissioner with the Government of India.

Plan 1.



R. W. Fisher.—The Vaccine Institute, Belgaum.

Captain* W. F. Harvey, I.M.S., in the Punjab and Hutchinson in Bombay were in favour of the glycerine method of preserving lymph. From experiments, very carefully carried out, Harvey[2] and Hutchinson[3] showed that glycerinated vaccine lymph was to be preferred to lanoline. The arguments for and against lanoline may be stated :—

Lanoline has no effect in reducing the extraneous micro-organisms, which are always present, in large numbers, in lymph collected from the calf. Owing to the presence of these large numbers of extraneous germs, badly inflamed arms are very often met with after vaccination with lanolinated lymph. Lanoline merely forms a convenient vehicle in which the crude pulp may be ground-up and filled into tubes for distribution, so that, whatever extraneous organisms are present, when the pulp is collected from the calf, will remain alive and active when the lymph is used for vaccination. Further, lanoline tends to become acid in India, and acidity in vaccine lymph is fatal to the living virus. Emulsifying the crude pulp with 50 per cent glycerine solution, on the other hand, has a very powerful purifying effect and almost all extraneous germs, except those which form spores, are rapidly killed at tropical temperatures. It was further held, by the advocates of lanolinated lymph, that the vaccine principle was preserved intact, in the case of lanolinated lymph, for a much longer period than was possible, in the case of glycerinated lymph, but experience has not borne out their contention, and lanolinated lymph does not seem to give nearly such good results in India as were expected of it by its advocates. Further, it has been proved by Noguchi[4], that tropical temperatures alone are sufficient to cause fairly rapid weakening of the vaccine principle, irrespective of the vehicle with which the lymph is mixed. In fact, he has shown, that vaccine lymph which has been dried, loses its potency in proportion to the increase of the temperature of the air. In the case of glycerinated lymph, if a strong strain of lymph is maintained and due care is taken in preparation and distribution, it has been proved that the lymph retains its potency sufficiently long to obtain very good results in the hands of vaccinators, working over very extensive areas of distribution, such as the Provinces of Bombay and the Punjab.

Proceeding in the light of these ascertained facts, Hutchinson in 1904 began gradually to replace the lanoline method by glycerine, and glycerinated lymph was supplied to all the vaccinators in 1907-08.

---

* Now Lieut.-Col. W. F. Harvey, I.M.S., Director of the Central Research Institute, Kasauli.

At this time Hutchinson's work was very much hampered; sufficient funds, staff and suitable buildings, in which to prepare his supplies of preserved lymph, were all wanting. The work was begun and carried on, for some time, in a stable in Belgaum town. When he was able to show that his lymph was proving a success, further funds were forthcoming and some *kutcha* buildings were rented about two miles from Belgaum in August 1904. The Institute was then called Belgaum Vaccine Institute and its staff consisted of a Superintendent, three vaccinators and two servants.

The new Institute, in this way, fulfilled the double purpose of preparing a reliable vaccine lymph and serving as a training school for Inspectors of Sanitation and Vaccination and vaccinators.

The result of this system of training the vaccinating staff was at once apparent in improved results in the towns and districts which were worked by the trained men. Every effort was made, accordingly, to get as many of the vaccinators as possible passed through the Institute for training and in the very distant districts, such as those of Gujerat, the Inspectors only were brought for training and the Deputy Sanitary Commissioner of the District was able, with the help of these trained Inspectors, to instruct his staff of vaccinators at convenient centres. The results obtained since 1906-07 are shown in the following table :—

Year.	Primary Vaccination.		Remark.
	Case success rate.	Insertion success rate.	
1906–07	93·31	85·09	A fairly steady improvement will be observed.
1907–08	95·58	87·79	
1908–09	97·72	94·78	
1909–10	99·22	95·33	
1910–11	99·02	95·26	
1911–12	99·21	95·70	
1912–13	97·95	93·85	
1913–14	99·18	96·75	
1914–15	99·01	94·93	
1915–16	98·73	93·85	
1916–17	99·20	95·76	
1917–18	99·77	98·05	
1918–19	99·68	97·64	

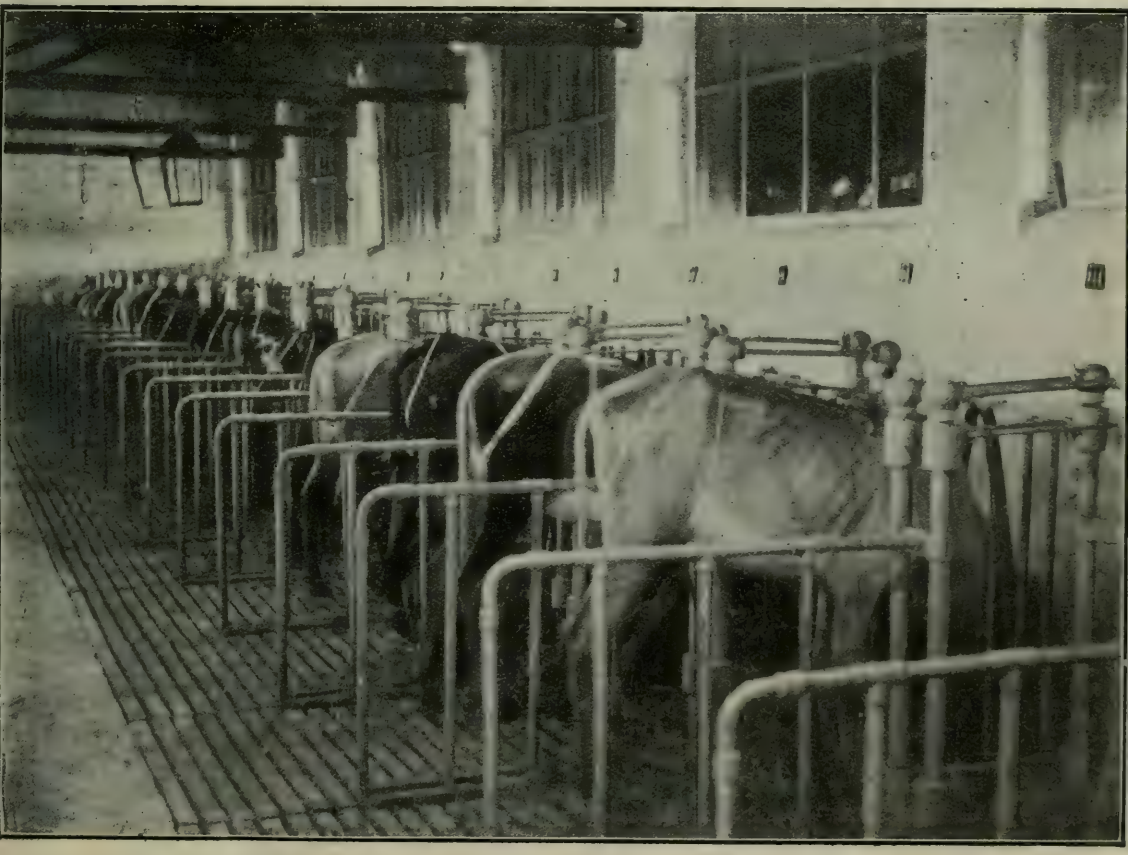
Encouraged by the success already obtained, Hutchinson drew up, and sent in to Government, a scheme for the acquisition of 176 acres and 35 gunthas of land about 2½ miles to the south of Belgaum. Plans were drawn up for the erection of a Vaccine Institute on the most modern

lines, at an estimated cost of Rs. 1,32,000, and the establishment of a suitable staff, at an estimated cost of Rs. 13,020 per annum in salaries. The scheme, with some minor modifications, was approved by Government,—funds were allotted, the land acquired and building of the new Institute was begun in 1907. The Institute was completed in 1910 and the work was transferred there in August of that year.

Method of Preparation and Purification of the Vaccine Lymph.

The supply of calves.—Calves are obtained from contractors of Belgaum. They supply all the calves required on a hire system. Rs. 4-8-0 per calf is paid for hire. The calves are carefully examined and selected on arrival at the dip enclosure. Unhealthy calves are rejected

Fig. 1.



Inoculated Calves' Stable.

also black-skinned animals and those showing more than two permanent teeth. They are from a year to 18 months old. Male or female are taken, but females are usually brought. Any calf showing signs of disease, after admission to the Institute, is returned to the contractor, who is paid Re. 1 as compensation. The disease which gives by far the most trouble is foot-and-mouth disease. When this ailment breaks out in a batch of calves, while they are in quarantine, it is usually necessary to return the entire batch to the contractor, as it is very infectious. Calves are kept in quarantine for 15 days and carefully examined on a table daily. When passed healthy, they are removed to the waiting stables where they are well-fed and carefully washed and groomed for a period, varying from 4 to 6 weeks, according to the demand for lymph and season of the year. They are passed through the dip every week. Batches of calves are selected for vaccination a fortnight before they are required, and these get special food and attention and their temperatures are taken daily, up to the time of vaccination. When selected, they are not further dipped. Any calf showing signs of illness is isolated and treated, and, if it does not recover in a few days, it is returned to the contractor. The cost, of feeding, per head, is Rs. 5-11-10 per mensem. Extensive grounds are available for grazing and exercise. Rock-salt is provided for the calves to lick. The selected calves are shaved, on the day previous to vaccination, and a large area of the belly and inner sides of the thighs is carefully denuded of hair. After shaving, the shaved area is dried and glycerine is applied. This tends to keep the skin in good condition. The average vaccinating surface, provided per calf, is 225 square inches. The calves are vaccinated in the cool of the morning, and, after vaccination, are kept in the inoculated calves' stable for 5 days when they are taken for the collection of pulp. After collection of the pulp, they are well dusted with powder and at once returned to the contractor ; in this way no risk is taken of these calves infecting waiting calves with vaccinia.

Buffalo calves have been used for lymph production, from time to time, but their use has been given up. The lymph obtained from buffalo calves is very impure and difficult to free from extraneous organisms. The lymph, further, has not proved so reliable as calf lymph. Buffalo calves cannot be obtained on hire in Belgaum.

Method of Vaccination.

The calf having been placed on the tilting operation table, the shaved area is thoroughly washed with soap and warm water, finishing off with

sterile filtered water. The area is then dried and covered with a sterile cloth. No antiseptics are used in the cleansing of the calf's skin.

Vaccination is carried out, with an ordinary scalpel, dipped in the seed lymph ; the scalpel is drawn lightly across the skin, making long, parallel incisions, into which the seed lymph is introduced by the back of the scalpel. The incisions should not be deep enough to draw blood, otherwise the resulting vesicles will not be so well formed. The lines of incisions are spaced about 3-8th of an inch apart. The vaccinated area is dried, by the aid of an electric fan, before removal from the table. The calf is then taken to the inoculated calves' stable, where it is kept for 5 days, while the vaccinia is maturing. Clean cloths are tied over the vaccinated part and renewed as required. Glycerine is applied to the developing vesicles 48, 72 and 96 hours after vaccination. This tends to prevent the premature drying of the vesiculation and helps its development. The calf is kept as dry and clean as possible, during this period.

Collection of Pulp.

At the end of 120 hours the vaccinated calves are again brought on the table and carefully washed with soap and warm water and sterile water and the area covered with a clean cloth. When thoroughly dry, the vesicles are inspected and a note made as to their quality. The quality is graded as good, fair or trial or rejected as unfit for collection. The pulp from those in good or fair class is not further tested as regards potency before issue. That from the ' Trial ' class is tried on children before it is declared fit for issue.

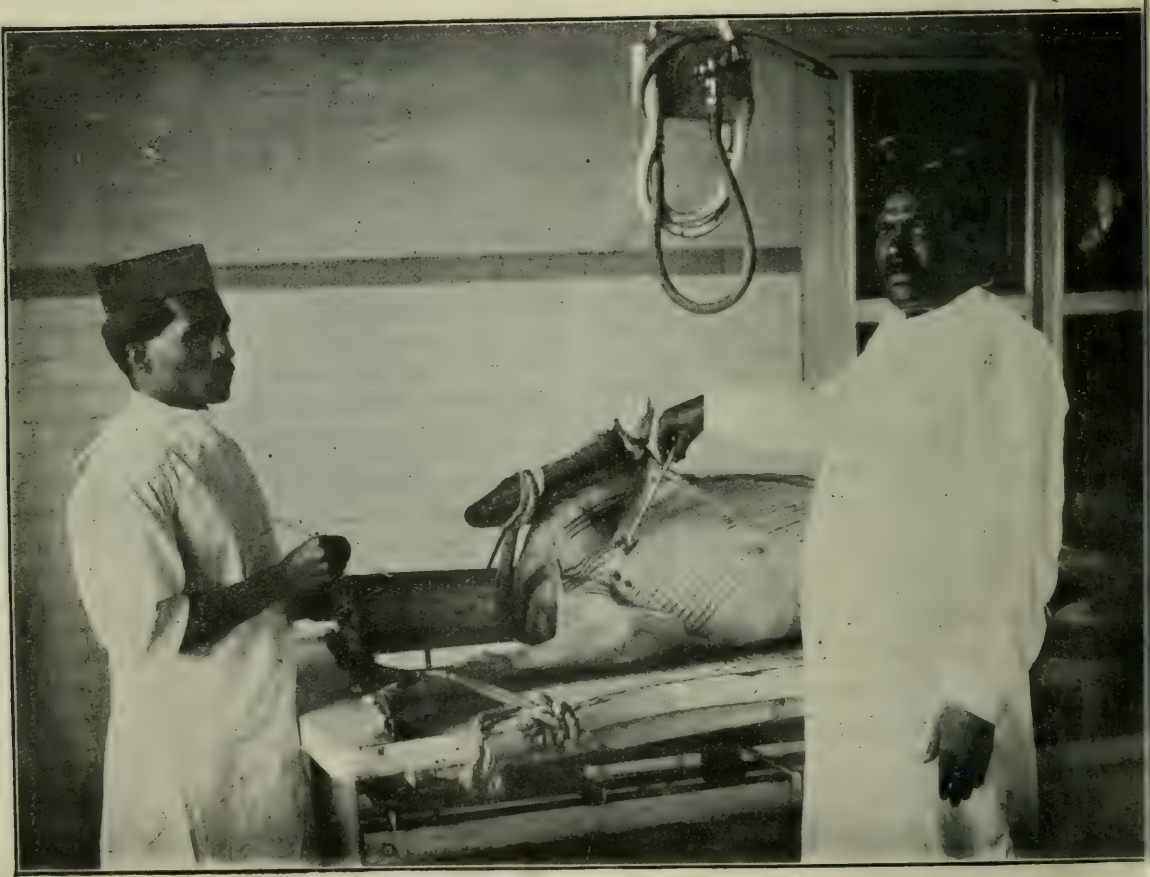
The percentage of ' Rejected ' calves is shown in the following table for a number of years :—

Table of Rejected Calves.

Year.	Percentage of rejected calves.
1910–11	23·49
1911–12	6·35
1912–13	3·35
1913–14	1·75
1914–15	5·62
1915–16	15·07
1916–17	10·42
1917–18	2·58
1918–19	4·74

The pulp is collected with a Volkmann's spoon and taken into sterile collecting bottles, the weight of each bottle being etched on the glass of the bottle. The pulp from a number of calves is mixed, so as to give about 50 grammes of pulp, which is found to be a convenient quantity

FIG. 2.



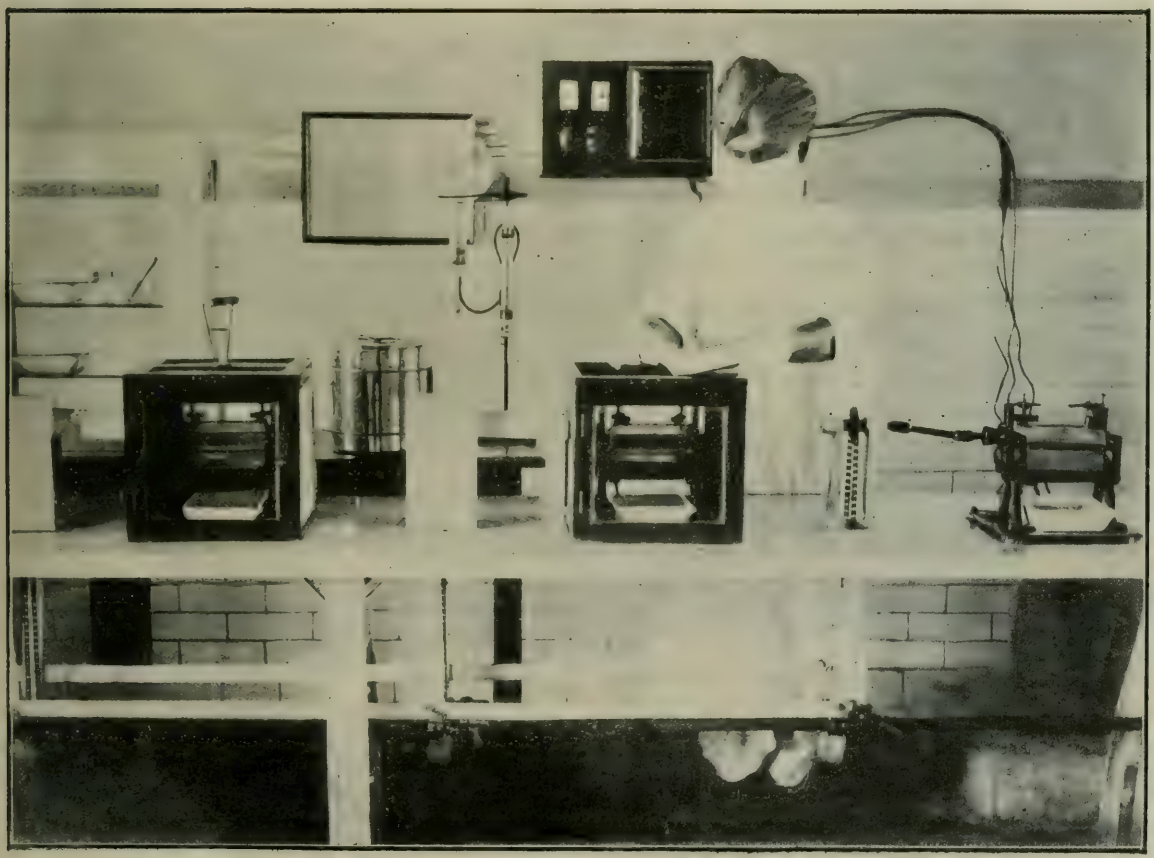
Matured Vesicles about to be collected.

to form a separate lymph number. This lymph number is dealt with, subsequently, so as to go through the mixing and purification processes without admixture with other lymph numbers and is passed for issue accordingly. The pulp from two or three calves usually forms one lymph number.

Mixture and Grinding of the Pulp.

The collecting bottles are now taken to the lymph mixing room and the pulp of each lymph number is carefully weighed and the requisite quantity of 50 per cent glycerine and water mixture is calculated, so that one part by weight of pulp is mixed with four times its weight of 50 per cent glycerine and water. The calculated quantity of glycerine and water is measured into a flask and is set aside with its corresponding pulp. Pending grinding, the pulp bottles are kept in an ice-box.

Fig. 3.



Grinding the Pulp.

On the same day, on which the pulp is collected, grinding takes place. Doering's lymph machines are used. These are formed by four glass rollers revolving in contact. The pulp and glycerine and water are

ground by passing through these machines four times, when a very uniform emulsion is obtained. Each machine is capable of grinding 20 grammes of pulp per hour. The glycerine and water mixture is prepared by mixing the best quality redistilled glycerine with sterile distilled water. The mixture is autoclaved, rendered faintly alkaline with soda bicarb., placed in a flask, which is held in a stand in an inverted position, connected by a 3-way tap to a graduated burette, and measured off into flasks as required. It is important that the lymph emulsion should be kept faintly alkaline, as any development of acidity is detrimental to the vaccine principle. The lymph emulsion, as it leaves the grinding machines, securely corked in suitable glass tubes, is found to contain a large number of extraneous micro-organisms. Ten milligrammes of this lymph, when mixed with agar culture medium and poured on plates, shows an uncountable number of these extraneous germs. They consist, for the most part, of staphylococcus albus, aureus and citreus from the calf's skin and seem to develop in the vaccinia vesicle at the same time as the vaccinia virus. They are not, as a rule, markedly pathogenic but, where crude unpurified lymph is used for vaccination, a number of abscesses are liable to be met with, and the inflammation, accompanying the development of the vaccine vesicle, is much more marked than in the case where lymph is used which has been rendered free from these extraneous organisms. Hence, the desirability of freeing the lymph from these germs. In many Vaccine Institutes, such as that at Hendon, London, reliance is placed on the purifying effect of glycerine, but this is found to be very slow, and may require many months' storage, where the lymph is kept at or below freezing point. Dr. Blaxall[5] states, in this connection, that glycerine has no germicidal action of itself, but in conjunction with heat, it is a powerful germicide and this action varies directly with the temperature. Hence, to get the purifying effect of glycerine on the extraneous organisms, it is found necessary to store the lymph at temperatures considerably above freezing point. Blaxall[5] showed this very clearly in his work at Hendon.

This method of purification was given a fair trial at Belgaum. I found it very uncertain in action, and a certain proportion of lymphs continue to show large numbers of extraneous germs, necessitating prolonged storage and frequent bacteriological examinations, before the lymphs are pure enough for issue. Unfortunately, prolonged storage frequently reduces the activity of the virus, so that trial on children is advisable before despatch to the districts.

Blaxall[6] has introduced the admixture of clove oil with glycerine and water mixture, in the proportion of ·1 per cent. He finds that the lymph, containing clove oil, is much more rapidly purified, so that a much shorter storage is necessary. No reduction in the potency of the lymph has been noticed, in his hands, by this method.

A trial of the addition of ·1 per cent clove oil was made at this Institute, but it was found that the action of the clove oil was very uncertain, in the case of the lymph produced here. The purification where clove oil was added, was more rapid than in the case of glycerine alone, but a certain number of lymph numbers proved very resistent and considerable numbers of organisms were still present after many weeks' storage. It seems a risky procedure to store a living vaccine containing an active disinfectant such as an essential oil. One would expect the active virus to be injured ultimately, and our experience of the method tends to confirm this to some extent, though, in view of Blaxall's work, its action must be very slow. Its use was, however, discontinued in favour of chloroformed lymph. Experiments were also made with glycerinated lymph, which was kept for a week or longer at about 50° F., till the extraneous germs were reduced sufficiently and then placed in cold store below freezing point. The results obtained by this method were not so good as in the case of chloroformed lymph.

In fact, the results obtained with glycerinated lymph, purified by chloroform according to the methods used at this Institute, have proved so excellent that all other methods have been given up for routine work. Rapidity of purification, by this method, is a great advantage, as experience has amply demonstrated that a certain proportion of vaccine lymphs, unless stored at very low temperatures, tends to decrease in potency the longer it is stored. So that, where lymph has been stored for any period over three months, it is found uncertain as regards its potency, and it cannot be issued, with safety, without previous trial on children to ascertain its activity.

Our experience at Belgaum is that, where a series of lymph numbers, collected from calves showing vesiculation of the same quality and treated in an identical way throughout the different processes, are stored under the same conditions, a small proportion of these lymph numbers will show marked deterioration, after four months' storage, while the remaining larger proportion will have retained its potency slightly, if at all, unimpaired. No cause, up to the present, has been discovered for this uncertain behaviour of stored lymph. It is probably due to the

growth of certain moulds or other resistent growths, which give off, in their growth, some chemical agent which is inimical to the vaccine virus The trial of all lymph, to test its activity prior to issue, would be the ideal procedure, but lack of sufficient children in the neighbourhood of the Institute, and the labour entailed, renders this difficult to carry out. In the case of Sind, only, it has been found feasible to await the result of a trial issue before despatching the remainder of that lymph number to Sind vaccinators. In this case it is essential, that a vaccine of the highest potency should be supplied, owing to the time taken by lymph packets in the post, before they come to the hands of the vaccinators, and also, owing to the very high temperatures recorded in Sind during the hot season. Therefore a preliminary trial issue is made to the Superintendent of Vaccination at Karachi, who sees, personally, the results obtained and at once advises us if the vesiculation is of good quality. In this way lymphs which do not prove resistent to these adverse conditions are identified and excluded from issue.

The Chloroform Process of Purification.

The removal of extraneous organisms from vaccine lymph, by the agency of chloroform vapour, was first introduced by Dr. A. B. Green[7] of the Lister Institute in 1902.

His method was to triturate the pulp with distilled water, one part pulp to three water. Chloroform-saturated air was then blown through this emulsion for two hours. The inlet and outlet of the vaccine tube were then clamped, and the chloroform retained in the emulsion for 18 to 42 hours. A current of sterile air was then passed through the lymph and the chloroform partially removed. The lymph was stored in an ice-chest at 10° C. and, immediately before issue, the remainder of the chloroform was blown off and glycerine was added, in the proportion of two parts of glycerine to the original weight of the pulp.

He found that all the extraneous organisms were killed off by this method and that the vaccine virus was still active, so that the lymph was ready for despatch without prolonged storage.

Blaxall informed me that this method of Green's was given a thorough trial at Hendon, but that it proved injurious to the vitality of the lymph in some cases, and he would only recommend it, where lymph was required so urgently as not to permit of storage.

The method seemed to offer great advantages in India, where the facilities for cold storage were very limited, and Hutchinson carried out a

series of experiments, with the object of so improving on Green's original method, as to overcome its obvious shortcomings. He argued that the chloroform was probably left too long in contact with the lymph, thus endangering the vaccine virus. Therefore, after chloroform had been passed for 2 to 3 hours, air was bubbled through the lymph and all trace of chloroform removed. He found that the extraneous germs were killed off by this method, so that plates poured from the chloroformed lymph showed no growth of the usual flora found in the crude lymph. The lymph, on the other hand, proved quite active and gave excellent results in the hands of vaccinators. It was found possible, by using this method, to have a pure lymph ready for issue in two weeks from the time of collection of the pulp from the calf, so that prolonged storage was unnecessary for purification purposes. Modifications of the process have been introduced in the light of experience and the objections, which were valid against the original method of Green, have been entirely overcome. The results obtained, with our chloroformed vaccine lymph in a tropical country, will bear comparison with those obtained in temperate climates, where more conservative methods of purification are still carried out. The method has stood the test of experience, and it is claimed that a vaccine lymph is issued from Belgaum, of a very high standard of potency, free from the usual flora of extraneous micro-organisms. Where sporebearing germs and moulds gain access to vaccine lymph, no known method will eradicate them which will not, at the same time, destroy the vital virus. But where proper care is taken in all the details of preparing the calves for vaccination, and collecting and handling the lymph, and sufficient staff is employed to enable this to be insisted upon, the introduction of these spore-bearing organisms can be reduced to a minimum, and, with the exception of tetanus, none of these resistent germs are found to have any pathogenic effect. Harvey[1] has stated that, with care, pathogenic organisms, such as those of tetanus and malignant œdema, can be absolutely excluded from the lymph, and Dr. Anderson,[8] Director of the Hygienic Laboratory, U. S. A., Public Health Service, records that vaccine for 2,000,000 persons has passed through the Laboratory and in no case has the organism of tetanus been detected, although specially searched for; and he further states that 'Where tetanus occurs after vaccination, the infection is received by a contamination of the vaccination wound such as may occur in the infection of any other surgical wound not properly cared for.'

A statement showing the results obtained with Belgaum lymph is given in Appendix A.

It must be borne in mind, when considering these figures, that every effort is made, in the districts concerned, to check results. Inspectors of Sanitation and Vaccination are on duty in each district and they carefully check the work of the vaccinators. These Inspectors are officials trained

FIG. 4.

The Chloroforming Apparatus

in Bombay, who have had to pass a searching examination and receive good pay. Their reports are, therefore, to be relied on. A Deputy Sanitary Commissioner is in charge of a number of districts and, during his tour of seven months of the year, he checks, personally, the vaccination work; and I am informed by these officers, that every reliance can be placed on the figures sent in. There are four Deputy Sanitary Commissioners

on duty in the Presidency proper and one in Sind, so that a very efficient supervision of the vaccinating staff is maintained. Further, it must be emphasised, in considering these results obtained with Belgaum lymph, that vaccination work is carried on throughout the hot season and not only in the cold weather, as obtains in some parts of India. A very severe test is thus thrown on the potency of the lymph, as, in many of the northern districts of the Presidency, very high temperatures are recorded during the hot months of the year, and the heat in Sind during the summer is notorious, shade temperatures of over 120° F. being often recorded.

Detailed Description of the Chloroform Process of Purification as practised at Belgaum.

The *apparatus* used:—

The apparatus consists of a water blast pump A, which is connected to a steady head of water, so that pressure varies as little as possible. The pump is connected by rubber tubing to a large empty bottle B, which receives any water accidentally driven over from the pump. The current of air then passes through an air filter C, consisting of a brass tube filled with cotton wool, the whole being sterilized in the autoclave. After passing through the filter, the air enters the gas washing bottle D, containing pure chloroform (B. W. and Company, anæsthetic). The chloroform-saturated air is now led through a Woulff's bottle E, containing dry sterile sand designed to interrupt any chloroform which might be driven over by accident. The air then passes into a chest F, which contains the tubes of lymph. This chest has a double jacket, the inner, made of brass, is so arranged that ice can be introduced into the box, ice cold water circulates round the jacket and a temperature of 10° C. can be maintained. The tubes G are large $1\frac{1}{4}''$ bore and arranged in series, with rubber tubing, so that the air is bubbled through each tube in succession and escapes at the end of the series through a tube led outside the box at H. At the beginning of the series an empty tube intervenes, so that any condensed chloroform is retained and does not gain access to the lymph-containing tubes. One-third of each tube is filled with lymph, and the glass tube, carrying the entering air, passes down close to the bottom of the tube, which causes the chloroform-saturated air to bubble up through the lymph and pass on to the next tube of the series. The pressure of air is so regulated that the air bubbles gently through the lymph without

carrying over to the next tube. Where more than one lymph number is dealt with at the same time, an empty tube intervenes. The temperature of the chest is important and great care should be taken to see that the correct temperature is maintained. The chloroform vapour should be passed while the interior of the chest and tubes are at 20° C. ; at this temperature the chloroform acts in such a way that a passage of vapour through the lymph for 20 minutes to half an hour is sufficient to remove most of the extraneous organisms from calf-lymph. Our object is *not* to remove *all* the extraneous germs and show no growth on agar plates, when subsequently the lymph is plated out. A few colonies should always remain, and these colonies furnish an accurate indication that the chloroforming has not been pushed so far as to injure the vaccine virus. When all colonies are killed, one cannot be certain that too much chloroform has not been used. If clean plates result from the plating of the chloroformed lymph, then the time of passing the vapour should be reduced, until a small number of colonies, say under 15, are shown on the plates. These few organisms are easily dealt with by the glycerine in the lymph, especially at the temperatures obtaining when the lymph is despatched through the post to the vaccinators in Indian districts. Experience has fully demonstrated that, if some organisms are found in the lymph after chloroforming, the vaccine virus remains uninjured. When the time necessary for passage of chloroform vapour has been determined by this method, such that a few colonies remain alive, as shown on agar plates, the vapour is passed for half that period and the blast pump is then closed and the tubes of lymph are reversed in the chest, so that the first tube becomes the last in the series and the last tube becomes the first. The pump is againt urned on and chloroform passes for the remainder of the time. This method of reversing the tubes prevents the lymph, receiving the vapour first, from being more acted upon than the end of the series, and tends to a uniform purifying process for all the tubes. When chloroform has passed for the correct time the gas-washing bottle is removed and air alone is blown through the lymph, the temperature of the chest being maintained for 10 minutes at 20° C. After this, ice is added to the chest and the temperature is lowered to 10° C. and maintained at this temperature while the air is being blown through. It is found that the passage of air for three hours removes every trace of chloroform from the lymph. The object of keeping the temperature at 20° C. for ten minutes is to prevent the possibility of the chloroform, dissolved in the lymph, condensing out as liquid chloroform, which might occur if the temperature

were at once lowered while the lymph was fully saturated. Liquid chloroform, coming in contact with the lymph, tends to kill the vaccine virus. The lowering of the temperature to 10° C. has for its object the prevention of injury to the virus by the action of glycerine at warm air temperature. In fact, all through, from the collection of the pulp until the lymph is finally sent to the post, every effort is made to keep the lymph as cool as possible, so as to retain the potency unimpaired by the effect of temperatures above freezing.

The great advantage of this method of removing the extraneous micro-organisms from lymph are obvious. The method, the details of which must be carried out with great care, has proved, after years of trial, perfectly safe, and, if the safeguards which have been described are attended to, no injury results to the vaccine virus. Lymph, by this method, can be rapidly and cheaply freed from extraneous germs, so that a pure product can be in the hands of vaccinators within three weeks from the date of collection. The results of vaccination on children, with chloroformed vaccine, are very satisfactory. Inflammation is reduced to a minimum, and, on the seventh day after vaccination, firm regular vesicles are obtained with a marked absence of inflammation in the surrounding tissues, the latter condition being the rule before the introduction of a germ-free lymph. An interesting experiment, with results bearing on the heat-resisting qualities of our chloroformed lymph, compared with lymph prepared by a number of other methods is worth putting on record at this point.

Three calves, showing vesiculation of fairly good quality, were selected and the pulp collected from each separately. These three pulps were each divided as follows :—

Two grammes labelled A, 2 grammes B, 2 grammes C and the remainder labelled D.

A was emulsified with four times its weight of normal saline containing 1 per cent phenol.

B was emulsified with four times its weight of normal saline containing 5 per cent phenol.

C was emulsified with four times its weight of normal saline solution.

D was emulsified with four times its weight of 50 per cent glycerine and water.

After emulsification 10 c.c. of D was taken and labelled D. The remainder was treated with chloroform vapour for 30 minutes and the

chloroform then blown off. This portion was labelled E. The five lymphs, now prepared as above, were examined by plating on agar and colonies counted.

The five lymphs, A, B, C, D and E, were then divided into three parts and labelled as follows :—

A 1, A 2, A 3, B 1, B 2, B 3, and so on.

The A 1, B 1, C 1, D 1 and E 1 were placed in the incubator at 37° C.

A 2, B 2, etc., were kept at room temperature, average about 24° C.

A 3, B 3, etc., were placed in cold-store, average temperature about 5° C.

After seven days' storage, as above, calves were vaccinated with each of these emulsions in separate lines of 3″ in length, so that calf No. 681 had three lines, representing the A series, three lines of B series, etc., in all 15 lines. This was repeated on two other calves. The calves were examined after 120 hours and the resulting vesiculation carefully compared and recorded; *vide* Appendix B.

The emulsions were then returned to the incubator, room and coldstore, as before, and kept for a further seven days and again a series of calves was vaccinated in a similar way and the results recorded. This was again repeated in further series of calves after 21 and 28 days' storage, but those emulsions, which gave no vesiculation, after 21 days, indicating the death of the virus, were not carried on further.

A study of the results, recorded in the Table, will show the very definite effect of temperature on the vitality of the vaccine principle. Whatever the diluent used, the virus is much weakened, and in many cases killed outright, after 14 days at blood heat. The very marked lethal effect of 1 per cent phenol at 37° C. is noticeable. Noguchi[4] found that his strain of vaccine was more resistant in dilutions of ·5 to 1 per cent phenol than in 40 per cent glycerine. My experiments do not confirm this; in 40 per cent glycerine the virus retained its vitality almost unimpaired for seven days at 37° C. and was still alive after 28 days. In 1 per cent phenol, on the other hand, the virus was killed after seven days and was barely alive after 14 days in ·5 per cent phenol.

The effect of heat on the lymph, which was prepared in saline solution, is very unexpected. The virus was killed after seven days at 37° C., except for one or two points which survived. The lethal effect must be accounted for by the marked multiplication of extraneous organisms which took place in the case of the saline-diluted lymph. The bacteriological results have not been included in the Table but are being worked out separately

it may be stated, however, that a very great increase of germs, as shown on agar plates, occurred in the case of the saline-diluted lymph, while in 1 per cent phenol, and the glycerinated lymphs, the organisms were much reduced after seven days' storage at 37° C. Evidently, the growth of these germs is very injurious to the virus, and, if lymph is not prepared containing an active germicide, such as phenol or glycerine, the virus will suffer when the temperature of storage is above freezing point. In Noguchi's experiments, already referred to, he worked with a germ-free lymph obtained by his intratesticular method. Such diluents, as saline distilled water and Ringer's solution, did not prevent the continued vitality of his virus at blood-heat ; so it is evident, that the injurious factor is the growth of extraneous organisms, which are always present in lymph prepared from the skin. This would account, also, for the rapid deterioration, which has been shown to occur in the case of lanolinated lymph, as the lanoline exerts no germicidal action and the extraneous germs are free to grow and, by the action of their toxins, infure the virus. These experiments are all in favour of glycerine as a diluent for vaccine lymph, as neither the glycerinated nor glycerinated and chloroformed lymphs showed the deterioration which was so marked in the phenolated and saline diluted lymphs. The vesiculation, in the case of the chloroformed lymph, was rather better than that given by the glycerinated lymph, while the vitality was very similar in both. The chloroformed lymph was rendered practically free from extraneous germs, the agar plates only showing 10 colonies, while in the case of the glycerinated sample, many organisms were present, but became rapidly reduced at blood-heat and room-temperature. In cold-store, the glycerinated lymph showed a slow but steady reduction of extraneous organisms 12 colonies being present in 10 milligrammes after 28 days' storage. The experiment, therefore, confirms the experience gained in the districts with chloroformed glycerinated lymph and ratifies the opinion, previously-formed, that glycerinated lymph, carefully treated with chloroform vapour, has very satisfactory heat-resisting qualities and can be depended upon to retain its potency, at blood-heat, for two weeks and at room-temperature for four weeks.

Purification by the chloroform method is under complete control, as the bacteriological test is a sure guide to the amount of choroform necessary to reduce the organisms always present in crude lymph. When dependence is placed on glycerine alone to purify the lymph, it is impossible to determine how long it will require for the glycerine to

reduce the extraneous germs sufficiently, to permit of issue. Certain lymphs purify quickly, while others are most resistant, and many months are often necessary before the germs are got rid of. In the meantime, the potency of the lymph has been endangered by prolonged storage, and such lymphs must be tried, to test their activity, before they can be issued with safety. Such uncertainty is very unsatisfactory and repeated bacteriological tests are required, entailing labour and expense. I am, therefore, strongly in favour of the method I have described for freeing vaccine lymph from extraneous micro-organisms and I can strongly recommend it to workers in the same field.

Storing, Tubing and Despatch.

After the lymph has passed through the chloroform process, office copies of each lymph number are taken in small 2 c.c. glass tubes, each corked with a sterile cork and dipped in melted paraffin. The rest of the lymph is filled into test tubes, corked securely and paraffined, leaving as little air between the lymph and the cork as possible. These separate lymph numbers are placed in wire baskets and kept in cold-storage with their corresponding office copies. When convenient, the office copies are taken out and agar plates are poured from each. Two loopfuls of lymph, weighing 10 milligrammes, are taken from each lymph number and mixed in a tube of melted agar culture medium at 42° C., well shaken up and poured into petri dishes. The plates are incubated at 37 °C. and colonies are counted after four days. Those plates showing 15 or less colonies are marked as passed for issue and entered in the despatch register accordingly. When more than 15 colonies are found, the lymph is stored for another week and again plates are poured from it. When colonies are reduced sufficiently, the lymph is passed for issue. It is rarely necessary to store lymph longer than a few weeks, as the large majority are ound pure enough at the first pouring to be passed.

Filling.

Lymph numbers are selected from the despatch register and the tubes taken from store and carefully decanted into a filling funnel. This is a glass funnel with a cover ; a piece of rubber tube is attached, which has a spring clip and glass nozzle. The glass funnel containing the lymph is put into an outer copper container (A), mounted on a tripod stand (B) and surrounded by crushed ice and the cover fixed. The rubber tube, with clip attached, projects from the bottom of the copper container and the Assistant, who has charge of the filling, is provided with a copper box (C)

containing the vials to be filled. These vials have been very carefully cleansed and plugged with cotton wool. They are placed in the copper box and sterilised by dry heat at 175° C. They measure 27 m.m. by 8 m.m. A vial is taken from the box and the cotton wool plug removed and $\frac{1}{2}$ c.c. of lymph is run into the vial from the glass nozzle by easing the spring

FIG. 5.

Filling the Lymph.

clip. The charged vial is then placed quickly in a wooden block, having holes to receive the vials. Another Assistant takes the vial and securely corks it. The corks are also kept in a copper box which has a perforated false bottom, each cork being placed head up in a perforation of the false bottom. They have been sterilised in position, by autoclaving, at a temperature of 125° C. for 45 minutes. The Assistant grasps the cork

by the head and introduces it into the vial and passes the corked vial on to a third Assistant who dips the cork and head of the tube in melted paraffin. The vial is then placed in a copper box with perforated false bottom and this box is pushed in gradually into a special metal box like a drawer (D). This metal box contains crushed ice. So the vials are always kept cold. These vials, containing ½ c.c. lymph, are issued as sufficient for 10 primary vaccinations, each child under one year receiving two insertions on each arm. Above one year, three insertions on one arm and two on the other is the rule with Government vaccinators. In the case of re-vaccinations, three insertions are given on one arm only. Capillary tubes were tried by Hutchinson but given up, as it was found that lymph did not retain its potency as well in capillary tubes, owing to the lymph being more exposed to the effect of temperature in the capillary tube.

Packing and Despatch.

The vials are now ready for packing for despatch through the post. Each vial is put into a rectangular piece of wood which has a hole bored in its long axis from one end, large enough to receive the vial. A piece of wool is placed at the bottom of the cavity of the wooden case, the vial is dropped in and the case is corked. The wooden cases containing the vials are packed in stout cardboard sliding boxes, each box large enough to contain four wooden cases. Along with the wooden cases a result statement form is packed in the cardboard box. The cardboard box is now placed in a large envelope, made of reinforced paper, and tied with string. The packet is then stamped and is ready for posting. A sub-post office, on the Institute premises, receives the packets. The result statement is returned to the Institute by the vaccinator correctly filled up and showing the insertion and case success. Cardboard boxes, wooden cases, and empty vials are also returned to the Institute.

While describing the method of despatch, something must be said regarding the difficulties which were encountered in dealing with Sind. Owing to the long distance of Sind from Belgaum, postal packets requiring a minimum of four days and maximum of nine to ten days to reach their destination in the Sind districts, together with the intense heat encountered on the railway journey from Bombay to Sind, in passing through the sandy tracts of that part of India, and the very high temperature recorded in Sind itself, it was felt that, under such adverse conditions, it would be asking too much of the keeping

qualities of the lymph to expect it to retain its activity when finally it reached the hands of the vaccinators. During three months of the winter season lymph could be safely transmitted, without encountering extremes of temperature, and would retain its potency long enough to give good results with Sind vaccinators. This short period, however, was not sufficient to allow vaccinators time to vaccinate the children

Fig. 6.

Packing and Despatching the Lymph.

in the areas for which they were responsible, and the objectionable and expensive practice of calf-to-arm vaccination would have to be resorted to during the remainder of the year, if some method could not be devised for counteracting the evil effects of the high temperatures on the potency of the supply of lymph from Belgaum. Thermos flasks were tried, but found too expensive in postage and difficult to pack to prevent

breaking,—also lymph despatched by this method would have to be sent in bulk to a distributing centre,—an unworkable arrangement. I have to thank Dr. Blaxall, Director of the Lymph Establishment at Hendon, for very kindly suggesting the method which was adopted and proved very satisfactory. This method consists of packing the wooden cases, containing the lymph vials, in small cane baskets. The wooden cases are surrounded by cotton wool, which has been saturated with water, and packed in the cane baskets. The lids are fitted and the whole securely tied with strong twine. A cardboard label is sewn on to the lid to receive the address and stamps. These baskets are made for me in the Ahmedabad Jail. They measure 5″ square, fitted with a lid. They are made of stout cane work and permit of evaporation of the moisture from the wet wool. It was found, by experiment, that a mass of wool of this size retained its moisture for about nine days in the hot weather, and during that time the contained lymph vial is kept quite cool by evaporation of the moisture. The hotter and drier the air, the cooler the packet becomes.

The improvement in the results from the Sind districts, when this method was introduced, was marked. Instead of an insertion and case success of 86·89 and 95·63, respectively, the results went up to 97 and 98.

In the case of despatch to Sind, it was found, further, that a certain small proportion of lymphs, which were classed as fair or good and which would return good results in the nearer districts, proved weak when exposed to the long journey to Sind. A system of trial was then introduced, as pointed out earlier in this paper. In addition, the Superintendent of Vaccination at Karachi came to Belgaum and studied, thoroughly, the methods of handling preserved lymph and was instructed in the precautions which are essential to success in carrying out vaccination. He, on his return, held a class for vaccinators of Sind, and with the co-operation of the Deputy Sanitary Commissioner, the vaccinating staff was well trained in modern methods. Much credit is due to the then Deputy Sanitary Commissioner, Major W. O'S. Murphy, I.M.S., for the way he persevered with the introduction of Belgaum lymph into Sind, and the results obtained to-day are largely due to his efforts. The insertion and case success, returned from Sind for the year 1918-19, were 97 and 98, respectively, and vaccination was carried on right through the hot season. It is claimed that the problem of carrying out successful vaccination with preserved lymph in the very hottest tropical climate, has been solved.

Further Remarks on Methods.

In the preparation of animal vaccine lymph, one of the many difficulties met with, in a tropical country, is the maintenance of a potent strain of seed lymph. If vaccinia is carried on from calf to calf, after a time the strain weakens in potency and the vesiculation obtained, after from six to eight moves on the calf, becomes very poor and many calves have to be rejected as unfit for collection. In the old days, in Europe, efforts were made to renew the stock by obtaining a fresh strain of vaccinia from natural outbreaks of the disease among cattle. This was an uncertain method, as the occurrence of natural vaccinia among cattle is a rare occurrence and hard to discover. Retro-vaccination, or the passage of the virus through children and back from the child to the calf, was found fairly efficient in maintaining the activity of vaccinia. The objections to this are obvious. Parents object to the collection of lymph from their children's arms, especially in a country, like India, where most of the population is uneducated and very suspicious of modern scientific procedures. The passage of calf lymph through buffaloes and back to calves, was found to keep the activity of the virus at a fairly high level, and this method is in use in many of the Vaccine Institutes of India. At Hendon, Local Government Board Lymph Establishment, the use of rabbits, for this purpose, was demonstrated to me by Dr. Blaxall and introduced in Belgaum. I find it an excellent method and, since rabbits have been used, no difficulty has been found in maintaining a seed lymph of high potency. The method in use is as follows :—

Some of the best vesicles, found on one or two calves at a morning's collection, are chosen and collected for use as seed lymph. The pulp is treated in the routine manner, being chloroformed and examined bacteriologically. This seed is used for vaccinating a further batch of calves, and again seed is collected from a calf or two which shows the best vesicles. After four or five moves on the calf, the seed is passed through a pair of rabbits. An area on the rabbit's side is carefully shaved and a non-pigmented area is selected for vaccination. Grey rabbits, so-called Belgian hares, are found the most suitable animals. The skin having been well washed and dried, the lymph is spread over the area selected and gently scratched with a piece of wood, such as a match. If incisions are made, with a lancet or scalpel, the results are very poor on the rabbit, but if the skin is well shaved, a few gentle linear scratches are sufficient. After 72 hours, vesicles will have formed and are removed by scraping over the surface of the skin with a scalpel.

The vesicles obtained on the rabbit are not formed like the vesicles found on a calf or child and often only a number of raised red papules are obtained. These, however, contain the active virus and must be scraped away with the sharp scalpel. The admixture of some blood in the scrapings does not matter and the material is rubbed up in an agate mortar with 50 per cent glycerine and water. The emulsion is then stored for use, and, when required, is used to vaccinate a calf. The result of the first move, on the calf, is usually good enough to be used for despatch. The best of the vesicles are chosen and collected for seed for calf vaccination. It is found that, after passage through the rabbit, the vesiculation on the calf is greatly improved, and gives firm, unbroken vesicles after 120 hours.

APPENDIX A.

Statement showing the results obtained with Belgaum lymph during the years 1914–15 *to* 1918–19 *inclusive.*

Year.	Primary Vaccination.		
	Number of children vaccinated.	Case success rate.	Insertion success rate.
1914–15	551,950	99·59	97·48
1915–16	683,305	99·05	94·85
1916–17	682,850	99·20	95·76
1917–18	674,954	99·77	98·05
1918–19	583,648	99·68	97·64

APPENDIX B.

Effect of diluents upon the survival of vaccine virus at different temperatures (pulp emulsified with four times its weight of diluent in each case).

Serial number of calf furnishing pulp for experiment.	Temperature at which emulsions A, B, C, D and E were kept.	Number of days kept at temperature in 2nd column after emulsification.	Serial number of calf vaccinated with A, B, C, D and E.	Results of lymph prepared in				
				1% phenol in normal saline.	5% phenol in normal saline.	Normal saline only.	5% glycerine solution.	50% glycerine solution subsequently chloroformed for ½ hour.
				A. 1	B. 1	C. 1	D. 1	E. 1
638	37° C.	7 days	681	—	++	0+	++	+++
,,	,,	,,	716	—	+	—	++	++
,,	,,	,,	724	—	++	—	++	+++
700	,,	,,	552	—	+	—	+	++
,,	,,	,,	728	—	+	—	000+	+
,,	,,	,,	556	—	+	—	00000+	+
656	,,	,,	749	—	00+	—	0+	000+
,,	,,	,,	734	0+	+	0 +	+	+
638	,,	14 days	727	—	00+	—	++	++
,,	,,	,,	538	—	0000+	—	+	++
,,	,,	,,	732	—	—	—	+	+
700	,,	,,	662	—	00+	—	—	00+
,,	,,	,,	660	0+	000+	0+	—	+
656	,,	,,	747	—	0+	0+	—	0+
,,	,,	,,	739	—	—	—	—	—
,,	,,	,,	755	—	0+	000+	00+	—
638	,,	21 days	661	—	—	—	—	00+
,,	,,	,,	659	—	—	—	00000+	00+
,,	,,	,,	657	—	—	—	0+	00000+
700	,,	,,	751	—	—	—	—	—
,,	,,	,,	741	—	—	—	—	—
,,	,,	,,	691	—	—	0+	0+	0+
638	,,	28 days	752	—	—	—	—	—
,,	,,	,,	748	—	—	—	—	—
,,	,,	,,	668	—	0+	—	000+	0+
700	,,	,,	756	—	—	—	—	—
,,	,,	,,	758	—	—	0+	—	—

The reactions are designed for a linear incision of 2½ inches as follows:—

Poor	+	Number of isolated points of vesiculation very weak ..	0+
Fair	++		
Fairly good ..	+++		
Gooo	++++	Unsuccessful ..	— —
Very good ..	+++++		

APPENDIX B - *contd.*

Effect of diluents upon the survival of vaccine virus at different temperatures (pulp emulsified with four times its weight of diluent in each case).

Serial number of calf furnishing pulp for experiment.	Temperature at which emulsions A, B, C, D and E were kept.	Number of days kept at temperature in 2nd column after emulsification.	Serial Number of calf vaccinated with A, B, C, D and E.	Results of lymph prepared in				
				1% phenol in normal saline.	5% phenol in normal saline.	Normal saline only.	5% glycerine solution.	50% glycerine solution subsequently chloroformed for ½ hour.
				A. 2	B. 2	C. 2	D. 2	E. 2
638	Room temperature.	7 days	681	++	++	++	+++	++++
,,	Average 23° C	,,	716	+	++	+	++	++
,,	,,	,,	724	++	++	++	++	+++
700	,,	,,	552	++	++	++	++	++
,,	,,	,,	728	+	+	+	+	++
,,	,,	,,	556	+	+	+	++	++
656	,,	,,	749	000+	0000+	0+	+	++
,,	,,	,,	734	++	++	0000+	+	++
638	,,	14 days	727	++	++	++	++	++
,,	,,	,,	538	++	++	++	++	++
,,	,,	,,	732	+	+	+	++	++
700	,,	,,	662	00+	++	0+	0000+	++
,,	,,	,,	660	0000+	00000+	0000+	+	++
656	,,	,,	747	+	+	0+	0+	+
,,	,,	,,	739	+	+	−	−	+
,,	,,	,,	755	+	+	0000+	0+	+
638	,,	21 days	661	+	++	++	+	+
,,	,,	,,	659	+	+	00000+	++	++
,,	,,	,,	657	+	+	00+	+	+
700	,,	,,	751	+	+	000+	−	00000+
,,	,,	,,	741	0000+	+	00+	−	+
,,	,,	,,	691	+	++	−	−	+
638	,,	28 days	752	−	−	0+	00000+	+
,,	,,	,,	748	+	+	0000+	+	+
,,	,,	,,	668	+	+	000+	+	+
700	,,	,,	756	+	+	+	−	+
,,	,,	,,	758	+	++	0000+	0+	++

The reactions are designed for a linear incision of 2½ inches as follows :—

Poor ..	.. +	Number of isolated points of vesiculation very weak ..	0+
Fair .	.. ++		
Fairly good	.. +++		
Good ..	.. ++++	Unsuccessful ..	..
Very good	.. +++++		

APPENDIX B—*concld.*

Effect of diluents upon the survival of vaccine virus at different temperatures (pulp emulsified with four times its weight of diluent in such case).

Serial number of calf furnishing pulp for experiment.	Temperature at which emulsions A, B, C, D and E were kept.	Number of days kept at temperature in 2nd column after emulsification.	Serial number of calf vaccinated with A, B, C, D and E.	Results of lymph prepared in				
				1% phenol in normal saline.	5% phenol in normal saline.	Normal saline only.	5% glycerine solution.	50% glycerine solution subsequently chloroformed for ½ hour.
				A. 3	B. 3	C. 3	D. 3	E. 3
638	Cold stores	7 days	681	++	++	++	++	+++++
,,	,,	,,	716	++	++	++	++	++
,,	,,	,,	724	++	++	++	++	+++
700	,,	,,	552	++	++	++	++	++
,,	,,	,,	728	+	++	++	++	++
,,	,,	,,	556	++	+	++	++	++
656	,,	,,	749	000+	+	++	++	++
,,	,,	,,	734	++	++	++	++	++
638	,,	14 days	727	++	++	++	++	++
,,	,,	,,	538	++	++	++	++	++
,,	,,	,,	732	+	+	++	++	++
700	,,	,,	662	++	++	++	++	+++
,,	,,	,,	660	000000+	000000+	+	+	++
656	,,	,,	747	+		+	+	+
,,	,,	,,	739	+	++	+	++	++
,,	,,	,,	755	+	+	00000+	++	++
638	,,	21 days	661	+	++	++	++	++
,,	,,	,,	659	++	+	++	++	++
,,	,,	,,	657	+	+	++	++	++
700	,,	,,	751	++	++	++	++	++
,,	,,	,,	741	+	+	+	++	++
,,	,,	,,	691	++	++	++	++	++
638	,,	28 days	752	+	+	000000+	+	++
,,	,,	,,	748	++	++	++	++	++
,,	,,	,,	668	+	++	++	++	++
100	,,	,,	756	+	+	+	+	+
,,	,,	,,	758	++	++	++	++	++

The reactions are designed for a linear incision of 2½ inches as follows :—

Poor ..	.. +	Number of isolated points of vesiculation very weak ..	0+
Fair ..	.. ++		
Fairly good	.. +++		
Good ..	.. ++++	Unsuccessful	—
Very good	.. +++++		

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SOME NOTES ON THE ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE IN MESOPOTAMIA, AND ON THEIR RELATION TO DISEASE.

PART IV.

SOME MESOPOTAMIAN NEMATOCERA OF ECONOMIC IMPORTANCE.

BY

MAJOR W. S. PATTON, M.B. (Edin.), F.E.S., I.M.S.,

Director, Pasteur Institute of Southern India.

ILLUSTRATED

BY

EDITH M. PATTON.

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IN this part of these notes, a few of the more important Nematocera of economic importance will be described: they are, however, incomplete, as it is impossible to record most of the Culicidæ which were collected, as the specimens were so damaged that identification is now impossible. The Culicidæ, however, are by no means so plentiful as may be supposed: indeed they are often conspicuous by their absence and are more or less localised in certain parts, where at times they become plentiful.

ORDER DIPTERA: SUB-ORDER ORTHORRHAPHA: NEMATOCERA: FAMILY CHIRONOMIDÆ: SUB-FAMILY CERATOPOGONINÆ: BITING MIDGES.

It will be readily understood that in a country like Mesopotamia extensive areas of land adjacent to the rivers are in the early spring

converted into vast shallow marshes ; and this is especially so in the case of the Euphrates. The flood water, which varies in volume in different years, depending on the amount of melting snow in the mountains where the rivers rise, breaks through bunds and banks and inundates the country for miles around. The water engineer will in the future doubtless conserve this water, and direct it into proper channels for purposes of irrigation, and not allow it to be wasted as it is at present. These marshes soon become covered with reeds and rushes, and afford excellent breeding grounds for the Chironomidæ. Seepage, which regularly takes place at flood time, leaves large patches of water just beyond the banks, and these too soon become overgrown with vegetation. Those who were encamped on the edges of these marshes and near the banks of the Euphrates during the flood season, will recall the immense swarms of midges which invaded the tents, and were an intolerable nuisance. In one particular camp at Khamisiyah, every day at dawn and sunset during the months of April and May a cloud of midges rose from the marsh and descended on the camp. They entered the tents in large numbers, and crawled over one's person causing great discomfort, fell into food in large numbers, so that the clear soup for instance usually contained more midges than the customary pieces of vegetables. Those who experienced this plague may perhaps wonder why Moses did not use them to intimidate the Egyptians of old.

An examination of a large number of specimens from the swarm revealed the interesting fact that they consisted of at least a dozen distinct species, many of them species of Ceratopogoninæ and one species of *Anopheles*, *A. pulcherrimus*. This mosquito entered the tents with the midges, and at once began biting and continued to do so even in broad daylight. Several of the Ceratopogoninæ, particularly two species, were seen attacking the larger species of *Chironomus*. The myriads of dragon flies helped to reduce the midges. These insects, many of which were of large size, sat on the tent ropes in rows, every now and then darting after a midge.

Genus Culicoides Lat.

Culicoides mesopotamiensis, sp. nov. (Plate XXIII, Fig. 1.) Head, palpi and antennæ light brown. Thorax brown without any distinct markings ; depressed areas, which are characteristic of all the species

Fig. 1. Culicoides mesopotamiensis, sp. nov. ♀ X about 45 times.

Fig. 2. Tersesthes mesopotamiensis, sp. nov. ♀ X about 40 times.

of the genus, well marked. Abdomen light brown with greyish pubescence. Legs light brown, a pale band near the bases of the fore femora, and another better marked on either side of the tibio-femoral joint; mid legs with femora pale at bases and apices, tibiæ with a distinct pale band near the tibio-femoral joint; hind tibiæ pale with a dark brown band at junction of upper and middle third. All metatarsi and tarsi light brown. Wings spotted; costa with two well marked dark brown spots, one about the middle covering the terminal portions of the first and third veins. The second spot not so well defined. Between the two spots, there is a sharply defined pale spot, and two joining each other between the second dark spot and the apex of the wing. Other pale spots as in the drawing. The male is unknown.

This species was extremely common all along the river banks and creeks during the summer months. It is troublesome to animals, biting horses, mules and cattle chiefly on the under side of the abdomen. The larvæ were plentiful in the algal matter in all the pools, streams and creeks.

Genus Tersesthes, Townsend.

Tersesthes mesopotamiensis' sp. nov. (Plate XXIII, Fig. 2.) Head dark, eyes kidney shaped and widely separated by a light brown stripe. Antennæ light brown, short, consisting of 14 segments; the basal segment consists of a narrow ring of chitin attached to the sides of the antennal cup; the second segment is globular, the third is pegtop shaped; segments four to ten are more or less rounded in shape, and the apical segment is elongated and swollen. Segments three to thirteen inclusive are armed with long lateral spines in addition to the ordinary bristles. Palpi consisting of three elongated segments, the middle is fusiform and has a large sensory area on the inner side about the middle; it is armed with short sensory spines on minute tubercles. Mandibles and maxillæ with well marked serrated edges. Thorax dark brown without any markings, but provided with short hairs. Abdomen light brown, and elongated; the ovipositor consisting of two lateral narrow spatulate processes. These processes are characteristic of the species of the genus. Legs brown, metatarsi elongated; claws equal in length, and pulvilli absent. Wings clear except basal half of costa which is brown, covered with minute hairs and erect spines. First and third veins well marked, ending about the middle of the costa, and joined by a cross vein. Fourth and fifth veins forked, the

former close to the base of the wing. The male and early stages are unknown.

This species of *Tersesthes* is of peculiar interest, in that, though it possesses well marked serrated mandibles and maxillæ, admirably adapted for piercing the skin, it was never observed to bite. It came into the tents in large numbers during the heat of the day in the month of May, when it was extremely abundant in the camp at Nasiriyah. It crawled over the face and hands, into the ears and up the nose and even up the trousers and arms, causing much annoyance. Many specimens were closely observed; they moved over the skin, feeling it with their fore legs as if looking for a suitable place to bite; they would then fly to another spot and behave in the same way. It was well known to all the occupants of the camp, and though one or two said they had felt its bite, the majority were agreed that it was not a blood-sucker.

All the other known species of the genus are blood-suckers, their bites causing much irritation. It is possible this species chiefly feeds on animals, and that perhaps in time it will become a human pest. Further observations on this midge would be interesting.

Biting Midges and Diseases.

It is only quite recently that Kinoshita has drawn attention to a species of *Culicoides* in Korea as being the possible carrier of some unknown disease-producing germ. This observer states that the bites of this species cause fever in children. Townsend believes that two species of *Forcipomyia* in the Southern Andean Region are the carriers of the parasite of Oriental Sore (Utah) common in that country. It is quite possible that these midges harbour a natural *Herpetomonas*, which is passed out in their excreta and is rubbed into the bite, or makes its way through the skin. The writer has examined a large number of different species of *Culicoides* for flagellates, but so far with negative results.

In South Africa, Van Sachegham believes that the virus of horse sickness is transmitted by a species of *Culicoides*. The possibility of these midges being the hosts of disease-producing germs should be borne in mind.

The larvæ and pupæ of *Culicoides* may be destroyed in large numbers by clearing away all weeds, algal matter and decaying vegetation from the margins of streams, ponds, creeks, etc.

FAMILY PSYCHODIDÆ: SUB-FAMILY PHLEBOTOMINÆ: GENUS PHLEBOTOMUS, RONDANI: SAND FLIES.

Phlebotomus papatasii Scop. A relatively large pale yellowish grey species with a dark coloured fringe to the costa and hind margin of the wing. Abdomen clothed with more or less erect hairs.

Phlebotomus minutus Rondani. A smaller golden ochraceous species. Abdomen clothed with decumbent hairs.

The two Mesopotamian species can be readily distinguished by looking for the characters noted above with the aid of a hand lens.

P. papatasii is the commoner of the two, and is extremely abundant appearing early in March, decreasing, and almost disappearing, during the hottest months, to reappear again in large numbers in August, September, October and November, when it disappears entirely. *P. minutus* appears a little later than *papatasii*, and is never so common; it also disappears during the hottest months to reappear again in August and September. The flies pass the winter as larvæ as far as the writer could ascertain.

P. papatasii is one of the most serious insect pests in Mespotamia. Its extensive range and breeding grounds, its relative immunity from enemies, all favour its multiplication, and at the height of its seasonal prevalence it abounds in incredible numbers. It is a striking fact that if a camp is pitched well in the desert where no human beings have been or are living, it appears the first evening in the camp, and makes itself evident by its blood-thirsty habits. Under these conditions it must be feeding on the blood of some small mammal or reptile, such as desert rats (Gerbils) and lizards. It was frequently noted that certain camps were heavily infested, while others, even near by, were almost free. This has been noted by different observers for many other species. It is difficult to give an adequate explanation for this phenomenon, but all the facts the writer was able to ascertain seem to point to the breeding grounds of one locality being specially suitable to egg-laying and food supply of the larvæ. Our knowledge of the breeding grounds of the species of *Phlebotomus* is at present extremely vague and scanty; it is true many observers have found the larvæ and pupæ, but no one has found them in large numbers as one would expect in the case of a gregarious insect.

The breeding grounds of the two species in Mesopotamia must be extremely wide, for the flies are found everywhere. The writer

devoted a large amount of his spare time to searching for them, and though he was rewarded in finding a few larvæ and pupæ of both species, he was never able to locate them in large numbers. He, however, came to the conclusion that both species breed in cracks and crevices in the ground in almost any locality, and that these breeding grounds are quite independent of mud walls, etc.; indeed, in most of the situations in which the adults were abundant there were no mud walls. In these cracks in the ground the conditions of food supply and moisture are eminently suitable for the growth of the larvæ. In the camp at Khamisayah, already referred to, the writer's tent was about 100 yards from the edge of the marsh. The ground was hard and there were many cracks and crevices, but there were no mud walls anywhere within the vicinity. Yet *papatasii* was extremely abundant, and there was no doubt that it was breeding in the cracks and crevices in the ground.

Sand Flies and Disease.

Phlebotomus papatasii and *P. minutus* transmit the unknown parasite of Sand Fly Fever, a disease which is very prevalent in Mesopotamia. Those who suffered from it will recollect its crippling effects; it led to a great deal of inefficiency among the troops of the Mesopotamian Expeditionary Force in the late campaign.

In a recent note, the writer drew attention to a new hypothesis regarding the spread of the parasite of Oriental Sore in Mesopotamia. *P. papatasii* and *P. minutus* are both known to harbour a natural *Herpetomonas*, and the writer believes, as the result of his observations, that this parasite passes into the skin as a flagellate, where the sand fly is crushed on the skin in the act of biting; and this happens very frequently. The parasite then rounds up and multiplies in the skin. This hypothesis explains many of the obscure points in connection with the spread of the disease. The writer does not believe that an intermediate host such as the gecko is necessary as a reservoir for the parasite; and the fact that a *Herpetomonas*-like parasite has been found in the gecko may only mean that the gecko may also become infected with this parasite by eating the flies.

The Control of Sand Flies in Mesopotamia.

The efficient control of *Phlebotomus* on a large scale in Mesopotamia is, for obvious reasons, out of the question. Individual control should,

however, be practised. Repellants, though unsatisfactory, help to a certain extent to keep off some of the flies. The writer found the ordinary kerosine oil of the country the most efficient repellant, though, of course, it is objectionable to some people. Other repellants are vermijelli, eucalyptus oil, anise oil and mixtures of these with some fatty substance. The writer always wore socks at night and tucked his pyjamas into them, he then anointed his neck, wrists and hands with oil and sprinkled some on his pillow. It is useful to keep a light near the bed as this deters the insects to a certain extent. Trousers, not shorts, should be worn in the evening. It is also a good plan to spray dark corners in bath and other rooms with kerosine oil emulsion; this certainly destroys many of the flies and deters others from resting in such places during the day. The writer made one attempt to destroy the flies by spraying cracks and crevices with kerosine oil emulsion in a localised area in a camp where they were very troublesome. The experiment, though on a very small scale, certainly reduced the number of flies.

Family Culicidæ : Sub-family Anophelinæ : Genus Anopheles.

Anopheles pulcherrimus Theobald. A beautifully coloured silvery species. Legs speckled; the last three and a half or three and three-quarters of the hind tarsal segments silvery white. Costa with six black spots, the basal ones small.

This is by far the commonest Anopheles in Mesopotamia, and it abounds in all the marshy areas, particularly along the Euphrates. As was pointed out above, it always accompanied the swarms of midges which rose from the marsh like a cloud at Khamisayah; it at once entered the tents and began biting during daylight. It was breeding mainly along the shores of the marsh, but the writer found many larvæ well towards the centre of the marsh; it also breeds in channels, water-cuts and ponds.

Anopheles stephensi Liston. A dark species with speckled legs, but without any conspicuous bands, and the last tarsal segments of the hind legs entirely dark. Costa with six black spots.

This species is somewhat localised in its distribution, and is commonest in Busra and its environs, where it breeds in the many channels and water-cuts among the date-palms. The writer took a few specimens at Nasiriyah, but it appeared to be rare. It is the

most important Anopheles in Mesopotamia as it is the natural carrier of the parasites of malaria. Its exact distribution should be carefully worked out.

Anopheles sinensis Wiedemann. A large dark species with the costa almost entirely black except for two small white spots, one at the junction of the middle and apical third, the other near the apex of the wing. It is possible that this is the variety described by Major Christophers, I.M.S., under the name *Mesopotamiæ*.

The writer took a few specimens in the marshy areas along the Euphrates; it is not a common species.

Two other somewhat rare species, *Anopheles lukisii* Christophers, and *A. maculipennis* have been recorded from Mesopotamia.

As pointed out above, it is not possible to record the Culicinæ as the writer's specimens are too mutilated to permit of identification. It may, however, be worth noting that *Aedes calopus* was taken in several localities, such as Nasiriyah, Amara and Busra.

In the next and concluding paper, the writer will briefly note some miscellaneous arthropods from Mesopotamia.

SOME NOTES ON THE ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE IN MESOPOTAMIA, AND ON THEIR RELATION TO DISEASE.

PART V.

SOME MISCELLANEOUS ARTHROPODS.

BY

MAJOR W. S. PATTON, M.B. (Edin.), F.E.S., I.M.S.,

Director, Pasteur Institute of Southern India.

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IN this, the concluding part of these notes, the writer will briefly note some of the arthropods, other than the Diptera, which were collected and observed, and which may be of some economic importance.

Order Siphonaptera : Family Pulicidæ : Genera Pulex and Ctenocephalus : The Human and Dog Flea.

Pulex irritans L. Genal and thoracic combs absent : mesosternum very narrow, and the ventral edge markedly oblique. Hind coxa distinctly pear-shaped.

Ctenocephalus canis Bouche. Genal and thoracic combs present. Distinguished from its near ally *C. felis* by the more rounded head of the female, less marked in the male, and the shorter first and last genal spines.

These two species are the common representatives of the family in Mesopotamia. *Irritans* abounds in the early spring in the vicinity of Arab huts and villages. On one occasion, the writer was living in a camp

adjacent to a recently evacuated Arab enclosure, and when the walls were demolished *irritans* became a pest; anyone going near or into the area soon had his socks, putties and trousers covered with active hungry specimens. The writer devised a novel method of ridding the area of fleas. Sheets of the well-known sticky paper used for catching flies were wrapped round the bare legs of the writer and three ambulance kahars, so that the sticky surfaces were outermost. Armed with these protective gaiters, the writer and the kahars walked over the flea-infested area disturbing the earth, and in a very short time the sticky surfaces of the papers were black with entangled fleas. The papers were burnt and fresh ones applied. Fewer fleas were caught on these, and fewer still on the third set of papers. In this way practically all the fleas were destroyed and the area cleared of the pest. The writer would recommend this simple but efficient way of disposing of fleas left in a camp or house by previous occupants.

Ctenocephalus canis is common on the dog and the cat, but does not appear to become a pest. It is interesting to note that no case of canine kala-azar was seen or heard of in Mesopotamia.

Order Rhynchota: Family Cimicidæ: Genus Cimex: The Bed Bug.

The Oriental bed bug (*Cimex hemiptera*) does not occur in Mesopotamia except in Busra and Baghdad, where it has been introduced by Indians. In the purely Arab towns and villages it is unknown. This is a fact of some importance as several observers have recently maintained that kala-azar is endemic in Lower Mesopotamia. At Nasiriyah the writer made a very careful house to house enquiry in order to try and find out whether kala-azar occurred among the inhabitants, but he never saw or heard of a case. There can be little doubt that the cases which have been recorded from Lower Mesopotamia contracted the disease in India.

Order Anoplura: Sub-order Siphunculata: Family Pediculidæ: Genus Pediculus: Head and Clothes Lice.

Pediculus humanus capitis and *P. humanus corporis* are two common ectoparasites of the inhabitants of Mesopotamia, the head louse, perhaps, to a less extent than in India; the clothes louse is, however, quite common. There is nothing in particular to note regarding these pests.

Order Acarina: Superfamily Ixodoidea: Families Argasidæ and Ixodidæ: Soft and hard bodied Ticks.

Only the following representatives of this superfamily were collected in Mesopotamia: doubtless, they do not represent anything like the true tick fauna of the country.

Family Argasidæ.

Argas vespertilionis Latr. Quite a number of specimens of this species were collected at various times; they were chiefly found crawling about on tents, furniture, and on clothes; they had most probably left their hosts, a species of pipistrelle, when it rested in the tents at night.

No species of *Ornithodorus* was found by the author; this is rather surprising seeing that the dromedary is the host of *O. savignyi*. Warburton, however, records a new species from Upper Mesopotamia.

Family Ixodidæ.

Rhipicephalus sanguineus Latr. This species was very common on the local pariah dog. *Piroplasma canis* was found in the blood of one dog.

Boophilus annulatus Say. This species was extremely abundant on the local cattle, cows and buffaloes.

Hyalomma ægyptium. The dromedary was the chief host of this tick; almost every animal had many specimens between its toes, in the ears, and around the genitalia.

Hæmaphysalis bispinosa Neumann. A few specimens of this tick were collected off calves.

Hæmaphysalis flava Neumann. A few specimens were collected off dogs.

Before concluding these notes, the writer would like to take this opportunity of thanking Professor Bezzi for helping him in identifying the Diptera. He would also like to point out that Mesopotamia would be an excellent field for studying the habits, particularly the breeding habits, of *Phlebotomus*. This insect is one of the most serious pests of the country in that it is responsible for the spread of Sand Fly Fever, and probably of Oriental Sore in the way described in an earlier part of these notes; these two diseases cause much inefficiency. At the same time the two other pests, the Gad Flies and the House Flies, should not be forgotten, especially the latter.

The writer will always be glad to help anyone with the identification of any of the injurious arthropods referred to in these notes. He will also be glad to communicate with any observer who would care to collect, with a view to studying, any particular group or species.

In conclusion he wishes to thank his wife for the many excellent drawings which illustrate these notes without which they would be mere lists of species.

VACCINE LYMPH PRODUCTION, PREPARATION AND PRESERVATION.

(ALL-INDIA SANITARY CONFERENCE, MADRAS, NOVEMBER 1912.)

BY

LIEUT.-COLONEL W. F. HARVEY, M.A., M.B., D.P.H., I.M.S.,

Director, Central Research Institute, Kasauli.

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IT is not possible to consider such points as the production, preparation and preservation of lymph separately from one another Thus for the production of lymph we require to have lymph which has been already prepared, and probably also preserved for some time, and therefore production is not independent of preparation and preservation. At the same time while we recognise this dependence, it is convenient to consider our subject in more or less consecutive order. We may postulate ideal cases and treat production of lymph, for example, as if we had at our disposal a perfectly prepared and perfectly preserved vaccinating lymph. In the same way we may under the head preservation take up the consideration of the subject of potency, which implies a reference not only to ultimate efficiency, but also to modes of preparation and production.

These explanations will suffice to justify my sub-division of the subject.

Note.—This paper was published in the Proceedings of the Second All-India Sanitary Conference of 1912. It is now published in the *Indian Journal of Medical Research* as it was not very accessible in the former publication and as the Editorial Committee consider that it will be of advantage to have it republished.

I. Production.

I need not describe, except in general terms, the methods used in obtaining the raw product which is made up into vaccine lymph. In its production it is still necessary to use an animal of some sort. The animal itself is inoculated with vaccine lymph. The eruption which results is removed as a whole and made up into the finished product. Many factors are concerned in the development of the all essential eruption.

(1) *Animal used.*—Before the introduction of calf lymph, and indeed for some time after, the material obtained from the human vesicle was that used for the continuance of vaccination. Since the introduction of calf lymph, and with it the possibility of preservation in bulk, attempts have been made to obtain lymph from other animals than the cow calf. In the Punjab the buffalo has served the purpose of vaccinifer for many years. The results have been excellent both as regards quality and quantity, although it would be going too far to say that they showed a marked superiority over those given by the cow calf. Chauveau asserts that the horse is the animal most suited for the production of vaccine lymph. Huon of Marseilles concludes strongly in favour of an asinine vaccine—a recommendation originating I believe with Chaumier. He argues (1) that the potency of a bovine vaccine is greatest when the strain used for inoculation is donkey lymph : (2) that on the donkey itself bovine vaccine gives as a rule excellent results, and that still better are obtained where donkey lymph is used to inoculate donkeys : (3) that sub-passage in the cow calf sooner leads to deterioration in quality of the lymph than in the donkey. There seems to be a fairly general consensus of opinion in favour of the use of the animal. The point would be worth investigating in India.

I have tried the goat as a vaccinifer but with ill success. The vesicles which developed were apt to contain much fluid and very rapidly became purulent. The rabbit has been greatly used after the recommendation of Calmette, but owing to its size, it has been adopted rather as an animal for regenerating lymph than for its production on a manufacturing scale.

(2) *Race and age of animal.*—Many vaccine institutes lay very special stress on the race of cow calf used. This, no doubt, experience has shown to be important. As far as I have observed little or no perceptible difference exists between the results obtained with the cow calf of the Punjab and the buffalo calf. The age of the animal might be expected to lead to some difference in the yield of lymph. But I have found that the use of large sized buffalo calves of two years old proved an advantage rather

than otherwise because of the greater vaccinable surface afforded. Dr. Guentcheff in Sofia uses heifers of any age from one to four years. Of course the older an animal is the less manageable does he become. The monetary value too of the animal for other purposes than vaccination grows with age. This question of the cost of the vaccinifer is an important one in relation to the cost of production of lymph. The cost will—after initial expenditure on purchase and maintenance—be dependent on the method of disposal of animals which is adopted.

(3) *Disposal of animals.*—In the days of calf-to-arm vaccination, when the villager's calf was impressed for the purpose, only a very small number of insertions was made on the animal and a correspondingly small number of persons treated from it. The return of the calf to the owner involved him in no loss, nor did the operation upon the animal cause it much inconvenience. With the introduction of preserved lymph it became advisable to obtain as much material as possible from the vaccinifer. The operation of removal of lymph under these circumstances takes time to recover from and I think the best and kindest plan then is to have the animal slaughtered. Is the flesh of such an animal fit for food ? If it is saleable, then the price of sale will reduce materially the cost of purchase and maintenance and so reduce that of production. I had no difficulty in Lahore in disposing of these vaccinated calves. In Brussels, Henseval and Convent likewise find no difficulty in disposing of calves and rabbits used for the production of lymph—these being used for food.

(4) *Quantity of lymph obtainable from a vaccinifer.*—Some hold the view that a calf may be over-vaccinated. Such was the opinion expressed to me by Dr. Blaxall at the Local Government Board Station. Others apparently attach no importance to the view that the potency of lymph may be affected by attempts at increasing the productiveness of the calf. I found, on taking over the appointment of Deputy Sanitary Commissioner, Punjab, that the yield from vaccinifers was very low indeed. This was due mainly to the fact that there was no urgent necessity for a large yield. Preserved lymph at that time was not widely used as such. Its chief use was to start the vaccination of cow calves at the beginning of the vaccination season—the continuance of this being by means of the calf-to-arm method. By degrees the yield was increased to 5, 10 and 15 grammes whilst in the hands of Major Forster, I.M.S.,* it reached as much as 40 grammes from a good sized

* Now Lt.-Col. Forster, I.M.S , Sanitary Commissioner, Punjab.

buffalo calf. Now 40 grammes of crude lymph has added to it 4 parts of 50 per cent glycerine, thus giving, approximately, from a single buffalo calf, about 200 grammes of final product. I found that with care a vaccinator would vaccinate 40 persons with 4 insertions each from one gramme. Thus I calculate that from one buffalo calf some 8,000 persons may be inoculated. The amount of crude lymph obtained, and on which this calculation is based, is evidently nowhere near the outside limit of possible production by a vaccinifer. With special methods of vaccination, and using heifers of two to three years of age, Chaumier has been able to obtain an average yield of 700 grammes of crude lymph and a maximum of 900 grammes. He calculates on an ability to vaccinate 100 persons from one gramme of vaccine, and thus at a dilution of 4 parts of 50 per cent glycerine to one of crude lymph it becomes possible to inoculate (number of insertions not stated) 350,000 and even 450,000 persons from one animal. At such rates it would not take many calves to supply vaccine for a province.

(5) *Site and extent of inoculation.*—The site most usually chosen is the abdomen with extension in some cases to the inner aspect of the thighs. The extent of surface utilised seems however to depend on our idea of what the animal can stand and on such ideas as we may hold of over-vaccination. Chaumier, whose results in respect of quantity of lymph obtainable I have given above, has vaccinated since 1895 the entire trunk of the animal and his scarifications are so close together that we may call his method one of sheet inoculation. A happy medium between the usual method followed in India and this method would result in greater production. I used what I called a method of feathering of primary incisions to increase the area of vesiculation.

(6) *Season of inoculation.*- The effect of high temperature upon the potency of vaccine lymph will be specially considered in its proper place, but temperature has likewise, or appears to have, an effect upon the satisfactoriness of development of the vesicle upon the vaccinifer. Here in Madras, I think you are well-acquainted with the difficulty of obtaining good or sufficient material from your calves in the hot weather. In the Punjab, the vaccine depôt is removed to the hills in the hot weather and the results are naturally as satisfactory during this season as in the plains during the cold weather. Chalybaus in Dresden found that the vaccine produced during the cold season showed itself more powerful and was more durable than the vaccine of the warm season. Vaccination of calves during the months of June, July and August was given up by him,

The temperature of the stables was not allowed at any time to go beyond 16°—18° C (60°—64°F.) because a more elevated temperature accelerated too much the development of the vesicles and diminished the value of the pulp. The effect of light, apart from temperature, may be a factor influencing the production of lymph. The production would seem to be better in dark stables. Humidity of the atmosphere almost certainly has an influence upon the size of vesicles produced and so upon quantity of lymph obtainable.

(7) *Maturation interval.*—This varies a little with different animals, but the only rule to be observed as regards this is that the lymph should be removed when vesiculation is complete and before pustulation sets in. On buffalo and cow calves the limits of time to removal are five to seven days. The maturation interval appears to be shortened with prevalence of high temperature and diminution of humidity in the atmosphere.

II. Preparation.

The subject heading of this section is taken to include all matters connected with the preparation of the final product.

(1) *Manufacture of lymph from animal pulp.*—In the days of arm-to-arm vacination, desiccation of vesicular fluid upon ' points ' served to provide material for the continuance of vaccination for occasions when children might not be present to permit of the actual direct transference of the contagion. The method is one of preservation of lymph, but has also been used for the preparation of lymph for transmission. It does not, however, seem to me to offer any great advantages over the other methods of preparation in which fluid or semi-fluid substances are used to dilute the pulp. Of these we may consider especially lanolin, vaselin and glycerin :—

(*a*) Lanolin. This medium was introduced by Colonel King, I.M.S. It gave a lymph of convenient consistency. Lanolin lymph served a very useful purpose and still continues to do so. It enabled lymph to be diluted without interfering with its potency and so greatly increased the quantity obtainable from the calf.

(*b*) Vaselin was introduced as a medium by Colonel Bamber, I.M.S. As a diluent for crude lymph it did great service, particularly in bridging over the interval—the hot weather—between vaccination seasons. It was also used to some extent for general vaccination purposes. Vaselin and

Lanolin, while both of them sterile substances as obtained in commerce, exercise neither of them any inhibitory influence upon the bacterial content of vesicular pulp.

(*c*) Glycerin. This is extensively used now as a diluent of calf lymph all over the world. Its use for the purpose was first advocated by Dr. Monckton Copeman. The great advantage of glycerin is that it exercises a distinct influence on the bacterial content of the mixture without adversely influencing its potency as vaccine. The dilution which crude lymph may undergo whilst still remaining satisfactory does not seem to have been worked out very definitely. The point is an important one because, in times of stress such as those of epidemic visitation, it might be of great use to know exactly what dilution the lymph held in stock might be subjected to, that is, supposing there was insufficient time to produce a new supply of lymph for issue. But much more important than dilution of lymph pulp is the question of purification.

(2) *Purification.*—The crude material from which vaccine is prepared consists of the scrapings obtained in removal of the cutaneous eruption developed on the vaccinifer. Naturally, these scrapings contain innumerable skin organisms. It is these skin organisms which seem to give rise to the inflammatory reactions so often seen in the course of vaccination. To my mind such reactions are not evidence of activity of lymph but of its degree of contamination with extraneous organisms. My experience with the two forms of lymph which were used during the time that I had control of the special staff of vaccinators in the Punjab confirms that opinion. Some of the lesions produced by vaselin lymph—a lymph rich in extraneous organisms—were very severe. Crateriform ulcerations due to sloughing out of the centre of an insertion were common. I saw none such from the use of glycerinated lymph and this lymph is almost entirely free from the presence of bacteria. The association of absence of bacteria and absence of inflammatory reaction would appear to be a true one. Of the processes used in the manufacture of vaccine, those in which vaselin and lanolin are used afford no apparent purification of the product. Desiccation of lymph, undoubtedly, brings about a certain reduction in the bacterial content of lymph, but naturally effects no reduction of such organisms as resist desiccation. With the help of glycerin on the other hand an almost

complete purification of vesicular pulp can be brought about. The special point to be attended to in regard to this purification is that it shall not be carried too far, for unfortunately glycerin will in time attack the true vaccine virus. The process too takes time and requires also a certain temperature. The higher the temperature the quicker is the purification effected, but at the same time the greater is the liability to destruction of the vaccine virus. At low temperatures the purification is extremely slow. Thus, with glycerin as a purifying agent, we are unable to use the prepared vaccine immediately because of its impurity, and we are unable to preserve it under cold storage conditions because that itself interferes with the purification. And so we are liable to find that our glycerinated lymph at the time of use is impure or avirulent. It is difficult to steer satisfactorily between this Scylla and Charybdis. A solution of the difficulty, however, seems to have been found in using a rapid purifier of lymph which can be got rid of after its purpose has been effected and which can then be replaced by glycerin. The glycerin is then used only as a diluent and preservative. Such lymph may be placed without fear of retarding purification under cold storage conditions. The rapid purifier which possesses the property of leaving the vaccine virus unaffected by its action, and at the same time being removable from the mixture, is chloroform. The use of this re-agent was introduced by Doctor Alan Green. I tried it experimentally in the Punjab at the instance of the then Sanitary Commissioner, Colonel Bamber, I.M.S., and obtained satisfactory results. The procedure was then adopted as a routine measure. The advantages in using chloroform, which seem to me paramount, are that :—

(*a*) There is no delay in the utilisation of the lymph, which can be tried on the very day of its manufacture.

(*b*) The lymph after its rapid purification and after the removal of the chloroform can be kept at a low temperature (in ice chest or refrigerator) in glycerin admixture. Of course care must be taken to remove all traces of chloroform. The procedure consists in the passage through an ordinary lymph mixture of air saturated by previous passage through pure chloroform. One to two hours' passage is sufficient for reasonable purification. One of the difficulties to be overcome in this process is the prevention of formation of an excessive amount of froth. This can be done by making the delivery tube of wide bore. The chloroform is got

rid of from the mixture by detaching the intervening chloroform bottle and allowing sterile air to bubble through in place of the air laden with the chloroform. Voigt of Hamburg remarks with regard to chloroform lymph that he has only observed bad results from its use and he is very astonished to find in the sanitary reports of Western India that this vaccine gave in the Punjab in the years 1906-1908 positive results in upwards of 99 per cent of cases. He goes on to say that as no mention of the use of this vaccine occurs in subsequent reports the conclusion is that it is no longer used. But Lieutenant-Colonel Wilkinson, the present Sanitary Commissioner in the Punjab, tells me that chloroformed vaccine has been used and is still used in the Punjab. The results which I obtained in the years 1904 and 1905 were evidently still obtained in 1906–1908 and are presumably still obtained. Voigt looks at the question from the point of view of despatch of vaccine to Colonies situated in the tropics. According to him, both desiccated and lanolinated vaccine withstand a high temperature better than glycerinated vaccine, but lanolinated vaccine gives much better results than desiccated vaccine. My own experiments (comparatively few in number) instituting a comparison between lanolin lymph and glycerin lymph, both subjected for a short period to the temperature of the incubator (37°C), did not bear out the idea of the superiority of lanolinated lymph over glycerinated lymph. Doctor Martin, the Director of the Lister Institute, however, in a conversation also maintained the superior keeping properties of lanolin lymph. We may, perhaps, conclude by saying that further experimentation on the subject is desirable. It might for instance be preferable to mix lanolin with chloroformed lymph rather than glycerin.

(3) *Degeneration and regeneration of lymph.*—This subject may be discussed here as it involves the consideration to some extent of production, preparation and preservation, the three main sections of this paper. Now we may take degeneration of stock lymph to be a fact. It comes into the experience, we may say, of all institutes and measures have to be taken for rejuvenescence. When I visited the vaccine lymph station at Halle in Germany, I asked the Director what he did when his stock

lymph degenerated, and the answer was, I get fresh lymph from another institute. This is one way of getting over the difficulty, but gives no explanation of how lymph should be regenerated. Chalybaus, experimenting to determine how long sub-passage of lymph in vaccinifers might continue without degeneration setting in, succeeded in carrying the same strain of vaccine up to the 20th and even to the 22nd and 30th generations on cow calves. Nevertheless, he concludes as to the necessity of regeneration after the third sub-passage, either through the agency of the human subject or by using some other animal than the calf (horse, donkey, goat, sheep or rabbit) or by obtaining a new strain of vaccine from a variolous case—a variola vaccine. In the Punjab, I found that alternation from cow calf to buffalo calf, and *vice versâ*, served to keep the stock lymph virulent. Evidently this alternation of animals is useful for the regeneration of lymph. It is a method which was in use by vaccinators before the introduction of glycerinated lymph. Passage through the human subject was the mode of operation. A number of children with well-developed vesicles are collected together and vaccination done directly from arm-to-calf. The result of this inoculation is not very productive, but when the small amount of material so obtained is transferred to another calf, the eruption is less scanty and the lymph becomes regenerated. The rabbit, although a small animal, can be satisfactorily used for regeneration of lymph. But what is the cause of the degeneration ?

It may be due, in the first place, to the continuous use of one and the same kind of vaccinifer. Most probably more than one cause is concerned in the matter. Vaccine lymph does not consist of pure vaccine virus. There is in it a plentiful admixture of extraneous organisms. With continued sub-passages of these extraneous organisms they may gain virulence, whereas the virulence of the vaccinia virus may remain fixed. But with this increase in virulence of extraneous organisms we may have overgrowth, an overpowering of the specific virus and degeneration, not attributable to the vaccinifer used but to the contamination of the original lymph. Transfer this lymph before it is too late to an animal specially susceptible to vaccinia, and as the extraneous organisms are transferred to foreign ground, they may be rendered comparatively innocuous and the vaccine virus gain ground over them—become regenerated. Such, at any rate, is a possible explanation.

(4) *Stock lymph and issue lymph.*—By means of these terms we differentiate between the lymph which is used for the vaccination of

animals (stock lymph) and that which is issued for use in the protection of human beings against small-pox. As the production of issue lymph depends for its potency on that of the stock lymph, it is but common sense to say that the utmost care must be taken that the stock lymph shall be the best lymph available. For stock lymph we should take the best vesicles from the best eruption obtained. The lymph which has proved itself good stock should on no account be issued, but kept under optimum conditions for preservation. With such precautions the danger of unexpected degeneration will be warded off.

III. Preservation.

The preservation of the potency of any given lymph will depend to a considerable extent on the measures adopted for its production and preservation—the animal used, the character of the eruption, the diluent and preservative added to the crude material, the season of the year at which taken, and so on. These have been already considered. What we may discuss are the best means of keeping potent the manufactured lymph, the tests of potency and the methods of issue best calculated to preserve potency.

(1) *Maintenance of potency.*—A weak lymph, that is to say one which is derived from an unsatisfactory eruption on the calf, will not preserve well. Alymph likewise which has been taken after pustulation has set in is not likely to remain potent long. But apart from errors of production and preparation, the keeping qualities of a good lymph depend largely on its after treatment. One consideration alone seems all important in this connection and that is the temperature at which it is preserved. This must be as low as possible—refrigeration temperature indeed. To my mind this necessity is what gives the advantage to the method of purification by chloroform. There is no waiting for purification before the lymph may be passed into the cold storage chamber or ice chest. Stock lymph, which we have insisted must always be the specially chosen lymph, should only come out of the refrigeration chamber at the time of use. Issue lymph, unfortunately, has to encounter vicissitudes of temperature before its utilisation. In Lahore during the cold weather, so cold that ice making manufactories shut down, I still found it necessary to procure ice for the preservation of the lymph. It had to be procured from as far away as Karachi. If the chloroform process is not used, then it should be laid down that the lymph produced at a station be put under refrigerating conditions as soon as purification is reasonably far

advanced. The degree of purification necessary refers perhaps as much to the type of organism present as to the numbers. Lymph should always be certified free from streptococci. In the matter of inflammatory reaction produced by use staphylococci are by no means as important as the streptococci.

Conservation of vaccine lymph is a necessity for all institutes, and the longer that conservation can be satisfactorily carried the better the position of the institute as regards continuity of supply and preparedness for epidemics. Lymph must be able to be kept, if demands are to be met promptly. According to Chaumier, who repeated and confirmed experiments by Blaxall and Fremlin, a good vaccine lymph, placed in a refrigerating chamber at 10°C, will keep for a year, two years, or more. The attainment of such a low temperature, however, is by no means easy, but it may be laid down that the preservation temperature should not be allowed to rise above +10°C.

(2) *Standardisation of lymph.*—Vaccine lymph is very liable to deteriorate, and it is necessary to have some standard by which to gauge its potency at the time of preparation and again, if possible, before issue. An estimate of potency made at the time of preparation will give some idea of the length of time to which such lymph may be kept.

(*a*) Test at preparation. This may consist—as has been used by Major Forster, I.M.S.—in marking off on the prepared surface of the vaccinifer of a definite area with silver nitrate and inoculating this area in the same way as the remainder. I should recommend, as being still more precise, the use of a weighed quantity of the stock lymph for this area. The product of this area is weighed and the weight is an indication of the satisfactoriness of the results. The character of the vesiculation may also afford indication of potency. Another test—that proposed by Calmette and Guerin—consists in inoculating rabbits with serial dilutions of the lymph to be tested and observing in what dilution vesicles cease to be produced.

(*b*) Test before issue. This is best carried out at a vaccinating station. Such a station should be attached, if possible, to the institute itself. The number of insertions which prove successful is noted, as is also the character of the vesiculation. With linear scarification the production of on vesicles or only isolated vesicles on the line is indicative

of a poor lymph. A fimbriated margin shows a condition intermediate between poor lymph and that produced by the best lymph, namely, a continuous unindented margin. All results obtained with issue lymph should be capable of being traced back to its source, and so, by comparison of these results with those obtained with the same material in other hands, an idea will be obtained as to where a fault, if any, lies with the lymph or with the vaccinator. According to Chaumier, the quality of a vaccine subjected to test by means of the character of the vesiculation is best estimated on the third day. He considers that often the ultimate results of good, bad and indifferent vaccines may be very much the same.

(3) *Issue of lymph.*—All possible provision may have been made for the preservation of lymph while retained in the institute, but this may be nullified by its treatment after despatch. After issue the temperature conditions are not so easily controlable as before it. It is self-evident, however, that every thing should be done in issuing vaccine lymph to prevent the adverse influence of high temperature. Its issue, as I believe is done in some parts of India, in thermos flasks is a step in this direction. Its utilisation should on no account be delayed, as this increases greatly the chances of deterioration. I am doubtful whether any lymph used later than a fortnight from the date of its despatch will prove consistently satisfactory. Another circumstance which has a great influence on success or non-success of vaccination is the time of year at which the operations are conducted. Be the issue lymph good or bad and be the arrangements for transport to the vaccinator good or bad, the results obtained during the hot weather will never compare with those of the cold weather. Therefore all vaccination should be conducted during the cold weather months. In the Punjab, vaccination is carried out during the months of November, December, January, February and early March. The results are certainly satisfactory.

The main points of this survey may be set down as follows :—

(1) High temperature with attendant dryness of the atmosphere is inimical to the production of satisfactory vesicles by vaccinifers.

(2) Stock lymph must always be the best lymph obtainable and must be most carefully protected from influences leading to deterioration.

(3) Degeneration of the lymph supply appears to set in if one and the same type of vaccinifer be used.

(4) Refrigeration of a continuous type is essential to the preservation of both stock and issue lymph.

(5) Lymph which has been issued must be preserved from the action of high temperature and used as soon as possible.

(6) Vaccination operations should be confined to the cold season of the year.

BACTERIOLOGICAL AND LABORATORY TECHNIQUE.

BY

LIEUT.-COLONEL W. F. HARVEY, M.A., M.B., D.P.H., I.M.S.,

Director, Central Research Institute, Kasauli.

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INTRODUCTION.

THE series of articles commencing in this number cannot be strictly regarded as representing the results of research. If they prove helpful, however, to research and laboratory workers, they will serve a useful purpose. These articles, published now in serial form, to make them available, are part of a work on technique, of dictionary form, which, although all the materials for its preparation are in readiness, will take several years to complete. Some commentary on its form is required to make the arrangement of subjects and the method of indexing intelligible. The three main ideas governing the arrangement are :—(1) the utilization of a form of description by definite categorical steps without parenthetical or explanatory interpolation, (2) the utilization of 'Notes' to supplement, by subsidiary description, the deficiencies of the main description, and to afford a commentary on the method, (3) the utilization of a specially detailed index as the means of guiding the worker to all the information he requires. As a rule a considerable number of methods is given under the same heading, thereby affording an indication of the extent and manner in which a worker may modify the technique to suit his own convenience. The index, besides being very detailed, has special features. A decimal system has been utilized for indicating the position in the work of the subject sought for. This method, which has been used for purposes of library cataloguing and for description of the characters of zoological genera and species, has not, so far as I know, been used for the purpose to which I have now put it.

The advantages of the method are numerous and of these the most important are that :—(1) it gives a reference, not merely to a page, but to a definite part of a page—a point of great importance where economy of space in the text is aimed at, (2) it can be compiled by the author before even the work goes to the printer. The manuscript index is identical with the printed index, (3) it allows of future additions very easily. The following is the explanation of the index method. The letter and the number before the decimal point refer to the letter, and the number under that letter, where the main subject is treated. The numbers after the decimal point, each in succession, show the division and sub-division of the main subject. Only four decimal places are used and of these the first three refer to true divisions and sub-divisions. The fourth decimal place is reserved for the figures which appear in the 'Notes.' The method appears complicated in description, but is very simple in practice. A few examples will afford a better explanation of its application than lengthy description. Thus, for 'Antigen, cholesterin' we have the figure **A3·82.** This means that under Antigen, at section No. 3 of the 'A' subjects, we find cholesterin treated at division No. 8 and sub-division No. 2. For 'Antigen rabies' we have the figure **A3·511,** which means that the reference required is to be found similarly under **A3** at division No. 5, sub-division No. 1, and further sub-division No. 1. A figure such as **A3·4124** signifies a reference to a 'Note' No. 4 of division 4, sub-division 1, and further sub-division 2 of the subject **A3.** Sometimes sub-division does not carry so far as the third place and yet a reference to a note is required. The fourth place, as already stated, is reserved for the 'Notes.' We indicate this want of continued sub-division by ciphers, *e.g.*, **A3·4002** signifies Note No. 2 to division 4 of subject **A3.** In this series of articles an endeavour is being made to select those which will be most immediately useful. They still bear the numbers, however, which they will bear, not in the complete dictionary treatise, but in a work of lesser scope and completeness. This accounts then for the use of apparently arbitrary numbers for the series. Thus the commencing article on Antigen bears the number 3 instead of 1. Where other letters such as S, M, etc., are used in the text, these refer to subsequent articles.

The work is not intended to be elementary or for beginners, but as a guide to workers who have already acquired a knowledge of technique. There are no illustrations which take up space. Their place is taken

to some extent by the multiplication of descriptions of possible methods. Certain abbreviations of constantly recurring terms or phrases are used. The following is a list of those requiring explanation :—

0·85 S.S.S. ..	0·85 per cent sterile salt solution.	Incubate used without other qualification.	Incubate at 37C.
1·5 per cent citrated 0·85 S.S.S.	0·85 per cent sterile salt solution containing 1·5 per cent sodium citrate.	Percentage or other dilution of a substance without other qualification.	Always aqueous solution.
0·5 per cent carbolized 0·85 S.S.S.	0·85 per cent sterile salt solution containing 0·5 per cent carbolic acid.	N-10 or similar figure.	1-10th 'Normal' or similar fraction.
T.W. ..	Tap water.	Test substance, test organisms, etc.	Substance, organism, etc., under test or to be tested, as distinguished from standard substance, organism, etc.
D.W. ..	Distilled water.		
T.T. ..	Test tube, test tubes.		
= ..	Equal to, equivalent to, etc.	Organisms.	
Alc. ..	Alcohol, alcoholic, in alcohol.	S. ..	Staphylococcus.
Abs. alc. ..	Absolute alcohol.	St. ..	Streptococcus.
R.T. ..	Room temperature.	Sp. ..	Spirochæta.
Sec. ..	Second, seconds.	D. ..	Diplococcus.
Min. ..	Minute, minutes.	M. ..	Micrococcus.
Hr. ..	Hour, hours.	A. ..	Amœba.
D. ..	Day, days.	B. ..	Bacillus.
Wk. ..	Week, weeks.	V. ..	Vibrio.
M. ..	Month, months.	Index name for specific organism.	Given usually under the name of the species and not the genus.
Yr. ..	Year.		
Sat. ..	Saturated.		
Vol. ..	Volume, volumes.		
Sol ..	Solution, solutions.		
Amt. ..	Amount, amounts.		
Grm. ..	Gramme, grammes.		
In. ..	Inch, inches.		
Syn. ..	Synonym, synonyms.		
Gram. ..	Gram's method, Gram's stain.		
G.P. ..	Guinea-pig, guinea-pigs.		
P.M. ..	Post-mortem examination, Perform post-mortem examination.		
S.N.F. ..	Supernatant fluid.		
M.L.D. ..	Minimum lethal dose.		

I have in conclusion to make my acknowledgments to the sources from which descriptions have been taken. It would not be possible to do so in all cases, and I have, therefore, to confine myself, with no intention of evading due acknowledgment, to the text books or works which I have used. These are by :—Abel, Allen, Bandelier and Roepke, Bard, Barthélemy, Bass and Johns, Beatty, Besredka, Besson, Beurmann and Gougerot, Blaye and Guggenheim, Bolas, Braun and Luehe, Browning, Browning and Mackenzie, Brumpt, Calmette, Carmalt Jones, Conn, Craig, Crofton, Cross and Cole, Dunnicliff, Emery, Eyre, Fantham Stephens and Theobald, Fred, Gilbert and Carnot, Giltner, Gulland and Goodall, Hall and Herxheimer, Harrison, Herschel, Hewlett, Hiss and Zinsser, Horder, Hutyra and Marek, Jackson, Jordan, Jowett, Kendall, Kisskalt and Hartmann, Kolle and Wassermann, Kolmer, Kraus and Levaditi, Langeron, Laroche, Laveran, Loehnis, Luys, Macé McIntosh and Fildes, Mallory and Wright, Manson, Moore, Mueller, Muir and Ritchie, Park Williams and Krumwiede, Patton and Cragg, Pollock and Harrison, Prescott and Winslow, Prowazek, Rosenau, Savage, Simon, Shenstone, Spitta, Stengel, Stephens and Christophers, Stitt, Strangeways, Sutherland, Weinberg and Séguin, Woodhead, Wright, Zinsser Hopkins and Ottenberg.

Acknowledgments are also due to :—The Medical Research Council Reports, The Field Manual, U. S. A., Standard Methods, U. S. A., St. Luke's Hospital Manual of Laboratory Technique.

Finally, an endeavour has been made as far as possible to exclude from the text references to the names of the authors of method. It would have been better still to exclude these altogether, but I felt that in the interests of the worker looking up a method, who would often consult the index with the author's name as the only clue to that method, it was necessary to include a certain number of authors' names.

A3 ANTIGEN.[1]

Notes.—[1]Material for inoculation as vaccine and production of anti-sera ; reagent in serum tests, etc.

A3·1 BACTERIA.

A3·11 STOCK VACCINE.—(1) Sow the pure organism from single colonies on a sufficient number of T.T. agar slopes. (2) Incubate 24 hr. (3) Make films from the cultures and stain by Gram for purity. (4) Pour, with sterile precautions, 0·85 S.S.S. over the surface of one of the agar slopes. (5) Shake to bring about suspension[1] of the culture. (6) Decant the suspension so made on to the surface of a second 24 hr. agar slope. (7) Shake to bring about suspension of the second culture.

(8) Continue in the same way with the other agar cultures, adding more salt sol., if necessary, until a sufficient amount of and sufficiently concentrated suspension has been obtained. (9) Decant into a vaccine bottle and affix a sterile rubber cap or decant into an ordinary T.T. (10) Shake by hand,[2] or in a mechanical shaker, until the suspension is homogeneous. (11) Standardize by turbidity (**S. 7·12**), hæmocytometer (**S. 7·33**), or film (**S. 7·35**) methods. (12) Sterilize (**S. 9·31**) in the water bath at 54 C. for 30 min. or longer, if necessary. (13) Allow to cool. (14) Add liquid pure carbolic acid to give a concentration of 0·5 per cent. (15) Test sterility by sowing a sample of suspension on an agar slope, incubating 24 hr. and examining for growth. (16) Test toxicity and absence of pathogenic spore-bearing anærobes by inoculation of 1 c.c. subcutaneously in a G.P. (17) Transfer a measured portion[3] sterilely to a suitable quantity of 0·5 per cent carbolized 0·85 S.S.S. to give the required dilution, or dilute directly the concentrated suspension to bring it to the strength required for use as vaccine. (18) Distribute[4] into bottles or capsules in quantities suitable for use.[5]

Notes.—[1]It may be necessary to detach the growth from the medium with a glass rod or platinum loop. Care should be taken not to break the surface of the agar in doing so. [2]The use of sterile glass fragments or beads in the bottle assist in rendering the suspension homogeneous. The T.T. should be sealed off for this shaking in the blowpipe flame. [3]It is convenient to have ready sterilized bottles plugged with cotton wool. To each of these is added with a syringe a fixed quantity, say, 20 c.c. of 0·85 S.S.S. followed by addition of 0·1 c.c. liquid pure carbolic acid to give a concentration of 0·5 per cent. The wool plug is then replaced by a sterile rubber vaccine cap. The neck of the bottle with the cap is plunged into sterile melted paraffin to seal it. The quantity of suspension to be added to the carbolized salt sol. has to be calculated and this volume, if appreciable, withdrawn, prior to the addition, by means of a syringe, from the total vol. of carbolized salt sol. The withdrawals and additions of fluid are performed with a syringe by puncture of the rubber cap which is smeared over to sterilize it with liquid pure carbolic acid or lysol. *For example :*—Given a vol. of salt sol. of 20 c.c. and a concentrated vaccine suspension of 2,500 million organisms per c.c. The strength of the vaccine required is, say, 100 million per c.c. The addition of concentrated suspension necessary will then be 0·8 c.c. Withdraw firstly 0·8 c.c. salt sol. from the 20 c.c. total vol. Add then the 0·8 c.c. concentrated vaccine suspension to the carbolized salt sol. [4]It is convenient to have in hand varying strengths of vaccine so as to give a sufficiently wide range of dosage. Two strengths are usually sufficient. If it is advisable to send out vaccine in the actual dose required to be used—say in a vol. of 1 c.c.—it may be necessary to keep in stock four strengths of vaccine representing a series of four graded doses. [5]If the vaccine to be used is a compound one, *e.g.*, tuberculin and streptococcus, the necessary quantities of each may be removed separately at the time of use from their receptacles with a sterile 1 c.c. syringe and added to a sterile empty capsule in which they are mixed and then withdrawn for administration.

A3·12 AUTOGENOUS VACCINE.—(1) Isolate the causative organism or organisms from pathological material obtained from the

patient. (2) Use the cultures so obtained to prepare vaccine, A3·11, for the patient.

A3·13 VACCINE, LARGE SCALE PRODUCTION.

A3·131.—(1) Sow the standard culture on T.T. slopes or agar plates to obtain single colonies. (2) Incubate 24 hr. (3) Examine the colonies and mark one which has a typical appearance and is large and well separated. (4) Subculture from this colony to an agar slope and a bouillon tube. (5) Incubate. (6) Examine the cultures macroscopically and with a hand lens. (7) Test the growth on the agar slope by the rapid macroscopic method on a slide with high titre specific agglutinating serum. (8) Make subcultures from the broth tube, if the examination and the agglutination are satisfactory, on agar slopes=seed cultures. (9) Incubate 24 hr. (10) Add 1 c.c. 0·85 S.S.S. to each agar slope. (11) Suspend the growths in the salt sol. and decant the suspension obtained into large T. T. = seed suspension. (12) Make smears and stain by Gram as a test for purity of seed. (13) Use 3 per cent nutrient agar of suitable reaction. (14) Sterilize the requisite number of Roux bottles 1½ hr. at 170C. (15) Place 100 c.c. melted nutrient agar into each bottle. (16) Sterilize in the autoclave 60 min. at 120C. (17) Let the agar harden in position overnight. (18) Place in incubator 24 hr. on a slight slant. (19) Prepare 0·85 S.S.S. in flasks and in quantity sufficient for the making of stock vaccine suspensions. (20) Sterilize the salt sol. 90 min. at 120C. (21) Plant out 5 c.c. from each flask in freshly heated and cooled glucose bouillon. (22) Incubate 48 hr. (23) Reject the salt sol. if contaminated. (24) Prepare absorbent cotton wool swabs on wire rods for sowing.[1] (25) Inspect each Roux bottle for evidence of contamination. (26) Flame the plugs of the Roux bottles and remove them. (27) Flame the mouths of the bottles. (28) Remove water of condensation which has collected in the bottles with a sterile pipette. (29) Sow each Roux bottle with a well-charged swab from a tube of seed suspension. (30) Incubate 24 hr. with the Roux bottles on a slight slant. (31) Make plate cultures from the seed suspension left. (32) Examine each Roux bottle after incubation with great care and reject any which have atypical or contaminating growths, or which show impurities in films on Gram staining. (33) Add a suitable quantity of 0·85 S.S.S. to each flask to make stock vaccine suspension. (34) Leave the salt sol. for a few min. in contact with the growth. (35) Wash off the growth in each flask by means of the contained salt sol. (36) Decant the suspension obtained

into 500 c.c. flasks. (37) Cover the wool plugs with a cap of tin foil. (38) Place a lead quoit weight over the necks of the flasks and sink them[2] in the water of a water-bath. (39) Place in the water-bath a similar flask containing only salt sol., with thermometer inset, as control on the temperature. (40) Suspend a thermometer also directly in the water of the bath. (41) Sterilize the flasks in the bath 1 hr. at a temperature of 53 to 60C according to the organism used. (42) Remove the flasks and dry carefully. (43) Flame the neck of the flask thoroughly to a point somewhat lower than the uppermost level reached by the water of the bath. (44) Remove and standardize a sample of the stock vaccine suspension from each flask by turbidity (S. 7·12), hæmocytometer (S. 7·33), or film (S. 7·35) methods. (45) Calculate the amount of 0·85 S.S.S. to be added per c.c. to dilute the stock vaccine to the concentration required for use as vaccine. (46) Add liquid pure carbolic acid to the stock vaccine suspension to give a concentration[3] of 1 per cent. (47) Add liquid pure carbolic acid to the diluting 0·85 S.S.S. to give a concentration of 0·5 per cent. (48) Dilute the stock vaccine suspension in large sterile flasks with carbolized salt sol. according to the result of standardization and the quantity of stock vaccine. (49) leave 24 hr. at R.T. (50) Make cultures[4] from the carbolized vaccine in two bouillon culture tubes and one agar tube. (51) Incubate one bouillon tube and the agar tube aërobically and the other bouillon tube anaërobically to test sterility of the product. (52) Inject a G. P. intraperitoneally with 1·5 c.c., a white mouse subcutaneously with 0·5 c.c., and a rabbit intravenously with 1 c.c. of vaccine to test for sterility, spores of anaërobic organisms, toxicity of antiseptic and of vaccine, and antigenic power. (53) Observe the animals for effect of inoculation and test the serum of the rabbit 7 d. after inoculation for agglutination titre. (54) Fill the vaccine into suitably sized capsules. (55) Select at least 3 per cent of capsules at random and test their contents for sterility[5] in the same way as before bottling. (56) Make a second test of sterility if growth is found in any tube, taking a sample from twice the number of capsules as before. (57) Reject the vaccine if the second test shows growth. (58) Preserve the passed vaccine in a cool room or refrigerator and in the dark.

Notes.—[1] The sowing may be done by running in seed from a bouillon culture, covering the agar surface with the seed fluid and then emptying out into a dish with antiseptic the excess of fluid. [2] They should not be more than half full with suspension and should be sunk up to a short distance above the junction of globe with neck. [3] The 1 per cent carbolic acid concentration although greater than that used for vaccine does not appreciably affect the final concentration when the stock suspension is diluted. It

is often desirable to preserve the vaccine in the stock suspension condition, where immediate bottling is not necessary and in such a case it is an advantage to have the concentration of the carbolic acid preservative as high as 1 per cent. [4] This dilution may be done in various ways as :—(*a*) in a flask marked with upper limits of stock suspension and diluting fluid ; (*b*) by calculating the amount of 0·85 S.S.S. required to dilute the quantity of stock suspension to the requisite strength and mixing the two. In that case the quantity of stock suspension is approximately determined by the use of a dummy flask filled to the same level with fluid, which is afterwards measured ; (*c*) by removing measured quantities of stock suspension and adding to the requisite quantity of measured 0·85 S.S.S., as by means of a sterile syringe or graduated burette. [5] The reason why sterility is tested before bottling, as well as after, is that in some cases the construction of the bottles or capsules renders their subsequent use after being once filled difficult, and the test is applied with a view to saving the expense of a wasted bottling. If such a type of bottle is not used then only one sterility test need be applied—that after bottling.

A3·132.—(1) Use ordinary nutrient agar in T.T. (2) Dry over lime. (3) Examine each tube before it is selected for sowing, for sterility and easy removal of the wool plug. (4) Reject contaminated tubes, wet tubes, and tubes which have the cotton wool plug adherent to the glass. (5) Sow the tubes with one or two drops[1] of a 24 hr. bouillon culture of the organism. (6) Plant 50 or 100 tubes at a time, as a brew unit from one seeding of bouillon culture. (7) Incubate. (8) Examine cultures macroscopically and with a hand lens for atypical growths and contamination. (9) Add 4 c.c. 0·85 S.S.S. to each tube. (10) Suspend the growth by rubbing it down into the salt sol. with a sterile thin glass rod, slightly curved upwards at the point. (11) Pour the suspension out of the tube into a sterile glass filter funnel which has been placed in a 500 c.c. flask and which has been covered over with a sterile Petri dish. (12) Collect in the flask 200 or 300 c.c.[2] of suspension. (13) Standardize by turbidity (**S. 7·12**), hæmocytometer (**S. 7·33**), or film (**S. 7·35**) methods. (14) Place the flask with contents in a water bath previously raised to 58C.[3], along with a dummy flask filled with water and containing a thermometer. (15) Heat to bring the suspension to a temperature of 53 to 60C., according to the organism, and maintain at this temperature 1 hr. (16) Allow the flask to cool. (17) Dilute the suspension to the required strength. (18) Carbolize to 0·5 per cent with liquid pure carbolic acid. (19) Bottle.[4] (20) Test a proportion of the filled capsules or bottles for sterility, aërobically in bouillon and on agar, anaërobically in bouillon. (21) Store the vaccine in the ice-chest or in a cool place.

Notes.—[1]The seed culture is delivered from an inverted flask by means of rubber tubing and glass pipette. The latter has a capillary stem with the point slightly curved upwards, so as not to break the surface of the agar, and is used both for delivery of and for distributing the seed culture over the agar surface. [2]The containing flask will have been etched previously with a mark to indicate this level. [3]The temperature of the

bouillon falls slightly on introduction of the flask. [4]In the case of a compound vaccine such as 'T.A.B.' the separate constituents will be diluted to triple their vaccine strength and carbolized in their own separate flasks, after which they are decanted into a large flask which has been etched in three graduations to represent suitable and equal amounts of the three components.

A3·133.—(1) Subculture the stock cultures weekly on agar slopes so as to give single colonies. (2) Sow on agar slopes from single colonies to give seed cultures. (3) Wash off the growth with 20 c.c. sterile bouillon[1] to make a suspension.[2] (4) Add 4 c.c. of suspension to the agar in each seed bottle.[3] (5) Incubate 24 hr. (6) Add to the growth in each bottle 20 c.c. sterile bouillon.[4] (7) Shake to suspend the growth in the bouillon. (8) Make a separate culture from the suspension in each bottle on agar slopes to test purity. (9) Incubate the bottles containing the suspension for another 24 hr. along with the purity T.T. (10) Make smears from the suspension in each bottle and stain by Gram. (11) Examine the purity test agar slopes macroscopically and with a hand lens for atypical or contaminating growth. (12) Reject corresponding bottles if atypical or contaminating growths are found. (13) Distribute the seed suspensions from the bottles into 50 c.c. bouillon[1] in flasks, allowing the suspension of one bottle to go to two flasks. (14) Test a sample of suspension in each flask for purity by sowing and incubating agar slopes. (15) Sow vaccine agar bottles[6] by the addition of 3 or 4 c.c. of the seed suspension to each and distribute over the surface of the agar. (16) Add to each vaccine bottle after 24 hr. incubation about 20 c.c. 0·85 S.S.S.[1] from a 4-litre flask. (17) Shake each bottle to bring the growth into suspension. (18) Remove the wool plug from each bottle in turn. (19) Carbolize with pure liquid carbolic acid, on a swab, the outside and inside of the mouth of each bottle. (20) Flame carefully in the Bunsen flame. (21) Decant the contents of each bottle into single, large sized, numbered T.T. (22) Test a sample of the suspension from each large T.T. for purity by sowing agar slopes numbered similarly to the large T.T., and incubating 48 hr. (23) Sterilize by heating the T.T. with their contents 60 min. at 53C in a water bath. This is done before the result of the purity test is known. (24) Cool, and carbolize by the addition of 1-10th c.c. pure liquid carbolic acid to each T.T. to give a concentration of 0·5 per cent. (25) Mix thoroughly. (26) Examine the agar slopes already sown for purity of the suspensions macroscopically and with a hand lens. (27) Reject any T.T. the growth of which is atypical or contaminated. (28) Remove in turn the wool plugs from the large T.T. and carbolize the inside and outside of the mouth with pure liquid carbolic acid on a wool

swab. (29) Pour off the S.N.F. from the sedimented bacteria. (30) Decant the sediment from 17 to 18 large T.T. or roughly say 100 c.c. into a litre flask etched with a mark at 500 c.c. (31) Complete the vol. to 500 c.c in each flask with 0·85 S.S.S.[1] (32) Shake to mix and remove a sample for standardization by turbidity (**S. 7·12**), hæmocytometer (**S. 7·33**), or film (**S. 7·35**) methods. (33) Add 5 c.c. pure liquid carbolic acid to give a 1 per cent concentration.[5] (34) Leave for 24 hr. and then test for sterility aërobically in a bouillon tube and an agar tube, anaërobically in a bouillon tube. (35) Make a pooled sample of the entire brew and test for toxicity and spores of pathogenic anærobes by inoculation of 1 c.c. subcutaneously in a G.P. (36) Dilute with 0·85 S.S.S.[3] carbolized so as to give a final concentration of the mixture of 0·5 per cent[7] and at the same time the requisite concentration of b cteria as contained in the vaccine[8] for issue. (37) Bottle. (38) Examine the first and last capsules or vaccine bottle from each bottling flask by aërobic and anaërobic methods for sterility. (39) Preserve the vaccine in a cool dark place.

Notes.—[1]A sample of the bouillon or salt sol. is tested at the same time for sterility by sowing on an agar slope. [2]A sample is tested at the same time for purity of culture by sowing on an agar slope. [3]For large scale production of vaccine, nutrient 4 per cent agar in ordinary round quart bottles, in the form of roll culture of the medium is used. [4]In the case of cholera vaccine the previous heating to sterilize is omitted, and sterilization is effected simply by the addition of the carbolic acid. [5]It is often convenient to store vaccine for some time, before final dilution, in this concentrated form. A concentration of 1 per cent carbolic acid is useful as a preservative at this stage instead of the final concentration of 0·5 per cent. [6]The requisite amount of 0·85 S.S.S. is contained in 4-litre bottling flasks, into each of which the corresponding 500 c.c. of concentrated stock vaccine is decanted—with all the precautions of cærbolization and flaming of the mouths of flasks, described in certain of the previous operations. [7]This necessitates a little calculation, as the flasks already contain 500 c.c. concentrated vaccine carbolized at 1 per cent and this 1 per cent strength has to be allowed for. [8]If, however, the vaccine being prepared is only one of several constituents contained in the final vaccine, a further dilution has to be allowed for—that of the final admixture. Thus any one component of a triple vaccine such as 'T.A.B.' would be diluted only to the extent which leaves it 3 times the *final* strength. Thus, for example, if the 'T.A.B.' vaccine is to contain 1,000 million typhoid, 500 million paratyphoid A, and 500 million paratyphoid B per c.c., the dilution at this stage of each component would be to a concentration of 3,000 million, 1,500 million and 1,500 million, respectively.

A3·134.[1]—(1) Use tryptic[2] digest bouillon (**M. 7·71**) which has been sterilized by autoclaving, but must not on any account have been overheated.[3] (2) Use a 20 per cent glucose sol. sterilized separately[4] 1 hr. at 120C. (3) Add[5] the glucose solution to the bouillon by means of a sterile syringe in sufficient quantity to give a 0·5 per cent concentration of glucose. (4) Incubate the glucose bouillon to test sterility.

(5) Sow, if possible, with a suspension of the organism required. (6) Incubate. (7) Make a film from the growth in each flask and stain by Gram. (8) Sow a sample from each flask on agar slopes, incubate, and examine growth for purity. (9) Standardize by turbidity (**S. 7·12**), hæmocytometer (**S. 7·33**), or film (**S. 7·35**) methods. (10) Sterilize the contents of the flasks in the water bath[6] 30 min. at 56C. (11) Add liquid pure carbolic acid to 0·5 per cent concentration. (12) Allow the flasks to stand 1 or 2 d. to let the bacteria sediment. (13) Remove[7] the clear S.N.F. by a vacuum pump[8] and replace by an equal quantity of 0·85 S.S.S. (14) Test sterility.

Notes.—[1]Used for an organism such as the pneumococcus. [2]The medium used is of some importance as there may result a precipitation of phosphates, and this will interfere with any standardization of the vaccine by a turbidity method. [3]Otherwise organisms such as the pneumococcus will show poor growth. [4]If sterilized with the bouillon the product is rendered unduly brown, even if the sterilization is done in a steamer at 100C. The glucose must be in solution before being subjected to sterilization. [5]There is little danger of entry of organisms even into a vertically open flask if the cotton plug has been flamed and carefully removed and the mouth of the flask well treated with pure carbolic acid. [6]With the heating, the growth of certain organisms tends to sediment. [7]If the peptone bouillon is not considered undesirable, its replacement by salt solution may be dispensed with. [8]A piece of copper tubing, which is easily sterilized, may be used, to lead the broth out of the flask into the receiving flask.

A3·135.[1]—(1) Use rabbit blood agar medium (**M. 2·255**) made 1·5 per cent acid to phenolphthalein, in 4-sided quart bottles. (2) Sow with 3 c.c. suspension of organisms in bouillon. from pure culture on rabbit blood agar—1 to 2 T.T. cultures to 1 bottle. (3) Distribute the seed over the surface of the agar. (4) Incubate 24 hr. (5) Examine the growth for purity—macroscopically. (6) Add 10 to 20 c.c. 1 per cent carbolized 0·85 S.S.S. to each bottle. (7) Suspend the growth in the salt sol. by shaking. (8) Pour the suspension from each bottle into single T.T. (9) Leave 24 hr. (10) Make a film preparation from the suspension in each T.T. (11) Test sterility of the suspension in each T.T. (12) Decant the contents of the T.T. which have passed the tests into flasks etched with a mark at 100 c.c., until this amount has been filled in. (13) Standardize. (14) Dilute[2] with 0·85 S.S.S. in 4-litre flasks to requisite strength.

Notes.—[1]Method for influenza vaccine. [2]It is an advantage to adjust as nearly as possible the amount of fluid added to the growths in bottles so that a concentration shall be obtained which will, in quantities of 100 c.c., be capable of being decanted in one operation into a 4-litre flask containing the requisite amount of diluent.

A3·136.[1]—(1) Culture the organism for sowing in bouillon for not more than 14 d. (2) Sow in 1 litre of bouillon contained in 3-litre

flasks of wide bottom and about 20 cm. diameter. (3) Place the flasks in a semi-dark room on tables or shelves. (4) Keep at a temperature of 25 to 30C. for 6 wk. (5) Test purity of culture. (6) Sterilize the cultures in a water bath 15 min. at 55C. (7) Add liquid pure carbolic acid 0·5 per cent. (8) Bottle.

Notes.—[1]Preparation of plague vaccine.

A3·14 SENSITIZED VACCINE.

A3·141.—(1) Make a suspension of a 48-hr. agar culture in 0·85 S.S.S. (2) Mix with undiluted specific aglutinating serum. (3) Leave in contact 12 to 24 hr. (4) Remove the S.N.F., by centrifuging, if necessary. (5) Wash the deposit three times in 0·85 S.S.S. (6) Make a suspension of suitable strength for vaccine in 0·85 S.S.S. (7) Heat to sterilize 60 min. at 60C.

A3·142.—(1) Leave[1] the living organisms in contact with a sensitizing goat anti-serum.[2] (2) Use in this form as vaccine.[3]

Notes.—[1]Gonococci 12 to 24 hr. at 37C. and 12 to 24 hr. at R.T.; staphylococci and streptococci 1 hr. at 37C. and overnight at 0C. [2]About 1 c.c. to each 18-hr. T.T. agar culture. [3]Initial dose 20 million for staphylococci and streptococci, 200 million for gonococci.

A3·143.—(1) Make a suspension of an 18-hr. T.T. agar culture in 0·85 S.S.S. (2) Standardize. (3) Sterilize 15 min. at 55C. (4) Prepare suspension by dilution to contain the requisite concentration of bacteria—about 5,000 million bacilli per c.c. (5) Mix with an equal quantity of rabbit immune serum. (6) Keep in the cold overnight. (7) Centrifuge till the S.N.F. is clear. (8) Wash the sediment twice with 0·85 S.S.S. (9) Suspend in fresh 0·85 S.S.S.

A3·15 DETOXICATED VACCINE.—(1) Dissolve the organism in N-10 sod. hydroxide. (2) Neutralize,[1] after solution, with N-1 hydrochloric acid. (3) Allow the precipitate[2] to settle, or centrifugalize it down. (4) Re-dissolve the precipitate in N-10 alkali after rejecting the S.N.F. (5) Re-precipitate with N-1 hydrochloric acid. (6) Repeat this process about 6 times to obtain a non-toxic S.N.F. and a detoxicated precipitate.[1] (7) Use the precipitate suspended in 0·85 S.S.S. as vaccine.

Notes.—[1]The supernatant toxic fluid when neutralized acts as a good antigen. The non-toxic precipitate also acts as a very good antigen. The toxin can also be removed by simply washing the precipitate repeatedly with a weak acid such as 0·5 per cent sod. phosphate containing 0·5 per cent carbolic acid. [2]Some organisms precipitate more readily from alkaline sol. than others, when acid is added. The gonococcus, streptococcus, pneumococcus and Pfeiffer's bacillus are precipitated very readily.

On the other hand B. friedlænder, B. typhosus and B. coli can only be precipitated with great difficulty.

A3·16 LIPO-VACCINE.

A3·161.—(1) Grow the organism in glucose tryptic digest bouillon (**M. 4·71**). (2) Test purity of growth. (3) Standardize. (4) Sterilize in the water bath 30 min. at 56C. (5) Add liquid pure carbolic acid to 0·5 per cent concentration. (6) Allow the flasks to stand 1 or 2 d. to let the bacteria sediment.[1] (7) Remove the clear S.N.F. (8) Dry the deposited bacteria in a drying oven at 53C. under a current of sterile air. (9) Grind the desiccated bacteria to powder. (10) Incorporate sterilely into cotton seed oil containing 10 per cent lanolin.

Notes.—[1]If the organisms will not sediment by standing they must be centrifuged down in sterile capped centrifuge tubes.

A3·162.—(1) Add 0·85 S.S.S. to 24-hr. agar cultures. (2) Suspend the growth in the salt sol. (3) Shake to break up clumps. (4) Standardize by turbidity (**S. 7·12**), hæmocytometer (**S. 7·33**), or film (**S. 7·35**) methods. (5) Sterilize by heating in a water bath 30 min. at 60C. (6) Add cresol 2 per cent. (7) Transfer to sterile capped centrifuge tubes. (8) Centrifugalize at high speed until the bacteria are deposited. (9) Pipette off the clear S.N.F. (10) Decant with sterile precautions the deposit in the centrifuge tubes into a sterile flask containing a quantity of glass beads. (11) Have in readiness sterile[1] bland oil such as cotton seed, olive, or sweet almond oil, containing 10 per cent anhydrous lanolin. (12) Add the lanolinated oil to the flask in the quantity necessary to give the concentration of bacteria required for stock vaccine. (13) Immerse flask and contents in water at 65C. (14) Remove and shake at intervals. (15) Connect up the flask with a powerful Geryk pump, with an interposed bottle containing calc. chloride and exhaust until clearing[2] takes place (16) Disconnect flask from pump. (17) Preserve as stock vaccine in a cool dark place. (18) Strain through sterile fine wire gauze, and dilute for use.

Notes.—[1]Sterilized 15 min. at 120C. [2]The clearing that takes place as the oil permeates the bacteria is very striking.

A3·163.—(1) Grow 20 hr. in large Petri dishes. (2) Scrape off the growth from the surface. (3) Transfer to a small Petri dish. (4) Dry *in vacuo* over sulphuric acid or calc. chloride. (5) Weigh in a sterile weighed glass dish. (6) Grind in a ball mill 24 hr. or longer. (7) Mix 20 per cent melted sterile[1] lanolin into one-half the vol. of olive oil required to give the final concentration of vaccine suspension, and add the mixture

to the contents of the ball mill. (8) Grind the bacteria and oil mixture for 7 hr. (9) Add gradually the remainder of the oil.

Notes.—[1]Sterilized 15 min. at 120C.

A3·17 VACCINE SPECIAL ORGANISMS.

A3·171[1] B. TUBERCULOSIS.—(1) Sow large shallow flasks containing 5 per cent glycerin alkaline broth with a culture of B. tuberculosis. (2) Incubate 7 wk. and sterilize. (3) Filter off the bacillary growth. (4) Triturate finely. (5) Make a suspension of the powder, 1-100 in D.W. (6) Add an equal quantity of pure glycerin. (7) Mix.

Notes.—[1]Bacillary emulsion. 1 c.c. of the preparation=5 mgm. dried tubercle bacilli.

A3·172 B. MALLEI.—(1) Prepare in a similar way to bacillary emulsion B. tuberculosis (**A3·171**).

A3·18 EXTRACTS.

A3·181—(1) Make a suspension of a 24 hr. agar growth with 0·85 S.S.S. (2) Heat 1 hr. at 60C. (3) Leave 2 d. in the incubator at 37C. (4) Filter free of bacteria to obtain extract.

A3·182.—(1) Make a suspension of a 4 d. agar growth with 1 per cent sod. hydroxide. (2) Leave 3 hr. (3) Acidify moderately with 1 per cent acetic acid, with shaking. (4) Allow sedimentation to take place. (5) Remove S.N.F. from the precipitate. (6) Wash the precipitate repeatedly with D.W. until the reaction is only faintly acid. (7) Place the precipitate on sterile filter paper. (8) Wash with D.W. until the filtrate is neutral. (9) Dry the precipitate *in vacuo* over sulphuric acid or calc. chloride. (10) Preserve in the dried state. (11) Dissolve the powder for use in 1 per cent sod. hydroxide.

A3·183.—(1) Make a suspension of a 24-hr. agar culture with 0·85 S.S.S. (2) Sterilize 1 hr. at 60C. (3) Keep 2 d. at 37C. (4) Filter through an unglazed porcelain or Berkefeld candle. (5) Add 0·5 per cent carbolic acid to the filtrate.

A3·19 EXTRACTS SPECIAL ORGANISMS.

A3·191 OLD TUBERCULIN.—(1) Sow flat bottomed 100 c.c. flasks containing a shallow layer of 5 per cent alkaline glycerin bouillon with B. tuberculosis. (2) Incubate 7 wk. with precautions against shaking. (3) Concentrate the contents of the flask over a water bath at 90C. to 1-10th their original vol. (4) Filter the concentrated product through a porcelain candle.

A3·192 OLD TUBERCULIN.—(1) Fill half full 100 c.c. flasks with 5 per cent alkaline glycerin bouillon. (2) Float portions of culture on

the surface of the medium. (3) Incubate 7 wk. (4) Concentrate the contents of the flasks over a water bath at 90C. to 1-10th their original vol. (5) Filter through an unglazed porcelain or Berkefeld candle. (6) Test[1] on a G. P. infected 4 wk. previously with B. tuberculosis.

Notes.—[1]0·2 c.c. should kill by subcutaneous injection within about 18 hr.

A3·193 OLD TUBERCULIN.—(1) Sow half-litre wide bottomed flasks containing 250 c.c. 5 per cent alkaline glycerin bouillon with B. tuberculosis. (2) Incubate 7 wk. with precautions against shaking. (3) Sterilise 1 hr. at 100C. (4) Remove the organisms by filtration through hardened paper. (5) Concentrate the clear filtrate over a water bath at 90C. to 1-10th its original vol.

A3·194 MALLEIN.—(1) Sow half-litre wide bottomed flasks containing 250 c.c. 5 per cent glycerin bouillon of reaction 2·9 per cent acid to phenolphthalein with a virulent[1] culture of B. mallei. (2) Incubate 5 wk.[2] (3) Test purity. (4) Sterilize by moist heat 30 min. at 100C. (5) Remove the organisms by filtration through paper.[3] (6) Pass the filtrate through an unglazed porcelain or Berkefeld candle. (7) Concentrate the clear fluid over a water bath at 76C. to 1-10th the original volume. (8) Add liquid pure carbolic acid 0·5 per cent.

Notes.—[1]Exalted by intravenous inoculation and passage in the rabbit. [2]In some methods of preparation the culture is incubated 5 m. [3]Otherwise the subsequent filtration through a porcelain filter would result in clogging with the slimy growth.

A3·195 MALLEIN.—(1) Sow half-litre wide bottomed flasks containing 250 c.c. 5 per cent glycerin bouillon of reaction 2·9 per cent acid to phenolphthalein with a virulent[1] culture of B. mallei. (2) Incubate 3 wk. (3) Test purity. (4) Sterilize by moist heat 30 min. at 100C. (5) Concentrate to 1-10th original vol. over a water bath at 76C. (6) Filter through filter paper. (7) Pass the filtrate in a thin stream into 30 times its volume of abs. alc. (8) Leave 24 hr. (9) Collect precipitate on a filter. (10) Dry the precipitate *in vacuo* over sulphuric acid or calc. chloride. (11) Use the dry mallein in a dose of 0·6 grm. in the horse.

Notes.—[1]Exalted by intravenous inoculation and passage in the rabbit.

A3·2 BACTERIA.

A3·21 SERUM REACTIONS.[1]

Notes.—[1]For serum reactions and production of high titre sera. Many of the bacterial vaccines, antigens and bacterial extracts also serve for these purposes.

A3·211.—(1) Use cultures[1] of appropriate age. (2) Make a suspension of the growth with 0·85 S.S.S. (3) Standardize[2] by turbidity (**S. 7·12**), hæmocytometer (**S. 7·33**), or film (**S. 7·35**) methods. (4) Dilute to a suitable concentration with 0·85 S.S.S. (5) Add liquid pure carbolic acid[3] to give a concentration of 0·5 per cent. (6) Place in the incubator[4] for 3 d. (7) Filter[4] through paper. (8) Keep for use in serum tests.

Notes.—[1]12 hr. gonococcus and B. coli : 12 d. B. tuberculosis ; 24 hr. other cultures. [2]The suspension may require centrifugalization at low speed to bring down clumps before proceeding to standardization. In certain tests, such as that of the opsonic index, in which there is a normal serum control, the only standardization required is to approximate turbidity. [3]This procedure may be omitted and the suspension used in the tests without sterilization by carbolic acid. It must be so used in tests (*e.g.*, bactericidal power) in which it is essential that the organisms of the suspension should be living. [4]Incubation and after-filtration may be omitted.

A3·212.—(1) Make a suspension of the bacterial growth in 1-1000 formalinized 0·85 S.S.S. (2) Dilute to standard strength with 1-1000 formalinized 0·85 S.S.S.

A3·213.—(1) Make a suspension of a 24-hr. agar culture in sterile D.W. (2) Sterilize by heating 1 hr. in a water bath at 56C. (3) Follow by heating 1 hr. at 80C. (4) Shake 24 hr. along with sterile glass beads to break up clumps. (5) Filter through paper and then through a Berkefeld filter[1] with a neutral reaction. (6) Sterilize by heating in a water bath 30 min. at 56C. on each of three successive days. (7) Add liquid pure carbolic acid to 0·5 per cent. (8) Add 1 part 9 per cent sod. chloride to each 9 part antigen to make isotonic. (9) Keep for use.[2]

Notes.—[1] Instead of filtration through a candle, centrifugalization at high speed to give a clear or slig htly opalescent S.N.F. will serve the purpose. [2] Used in complement fixation tests.

A3·214.[1]—(1) Add 10 c.c. 2 per cent antiformin to a 24-hr. agar culture to make a suspension. (2) Decant into a T.T. (3) Leave the suspension 30 min. at 45C. (4) Render neutral to litmus by the addition of 5 per cent sulphuric acid. (5) Add 5 per cent sod. sulphite to remove excess of chlorine. (6) Test for removal of chlorine by means of starch odide paper. (7) Keep for use.

Notes.—[1] Used in complement fixation tests.

A3·215.—(1) Make a suspension of a 24-hr. agar growth with 0·85 S.S.S. (2) Centrifugalize at high speed. (3) Pipette off the S.N.F. (4) Dry the sedimented bacteria *in vacuo* over sulphuric acid or calc. chloride. (5) Weigh. (6) Grind up the dry powder with crystals of sod. chloride

sufficient to give an isotonic solution with the quantity of D.W. required to give the desired concentration of antigen. (7) Add gradually the required amount of D.W. (8) Shake 24 hr. (9) Filter through paper and then through a Berkefeld candle[1] with neutral reaction. (10) Add liquid pure carbolic acid to 0·5 per cent. (11) Keep for use.[2]

Notes.—[1] Instead of filtration through a candle centrifugalization at high speed to give a clear S.N.F. will serve the purpose. [2] Used in complement fixation tests.

A3·216.—(1) Make a suspension of a 24-hr. agar growth with 0·85 S.S.S. (2) Decant into a sterile T.T. (3) Add an equal vol. of abs. alc. (4) Centrifugalize. (5) Dry the sediment *in vacuo* over sulphuric acid or calc. chloride. (6) Weigh. (7) Grind up the dry powder with sufficient sod. chloride crystals to give an isotonic solution with the quantity of D.W. required to give the necessary concentration of antigen. (8) Add the D.W. gradually. (9) Dilute this stock suspension for use.

A3·217.—(1) Precipitate a heavy suspension of bacilli in 0·85 S.S.S. with an equal quantity of abs. alc. (2) Centrifugalize at high speed. (3) Dry the sediment *in vacuo* over sulphuric acid or calc. chloride. (4) Re-suspend at time of use.

A3·218.—(1) Precipitate a heavy suspension of the organism in D.W. with an equal amt. of 95 per cent alc. (2) Centrifugalize at high speed immediately. (3) Re-suspend the sediment in alc. (4) Centrifugalize at high speed. (5) Continue the process several times with alc. and then with ether. (6) Free the final sediment of ether. (7) Dry the sediment *in vacuo* over sulphuric acid or calc. chloride. (8) Grind to a fine powder. (9) Re-suspend in 0·85 S.S.S. at time of use.

A3·219.[1]—(1) Use a 24-hr. broth culture[2] of the organism. (2) Shake well. (3) Add formalin to 0·1 per cent. (4) Shake well. (5) Place in the ice-chest. (6) Shake at intervals daily for 4 d. and replace in the ice chest. (7) Standardize by dilution with 0·1 per cent formalinized 0·85 S.S.S. to the opacity of a standard culture or suspension.

Notes.—[1] For agglutination test. [2] Use flasks half filled with veal bouillon as medium and sown with a 24-hr. culture which has been sub-cultured daily for 10 d.

A3·22 SERUM REACTIONS SPECIAL ORGANISMS.

A3·221 DANGEROUS ORGANISMS.—(1) Dip the cotton wool plug of the culture tube in pure formalin[1] and re-insert. (2) Fit a rubber cap on the tube over the wool. (3) Invert the tube. (4) Leave the vapour to act 2 hr. (5) Make a suspension of the growth with 10 per cent formalin. (6) Centrifugalize down the organisms in the suspension.

(7) Wash the deposit with 0·85 S.S.S. and make a suspension in 0·85 S.S.S.

Notes.—[1] Formalin does not penetrate well, especially through water. The water of condensation is drained off by the inversion of the tube.

A3·222[1] B. TUBERCULOSIS.—(1) Triturate thoroughly about 10 mgm. tubercle powder in a mortar. (2) Add gradually 1 per cent S.S.S. to make a paste. (3) Continue the trituration. (4) Add more 1 per cent S.S.S. (5) Allow the suspension to sediment 2 hr. (6) Pipette off the upper fluid into centrifuge tubes. (7) Centrifuge at low speed to bring down clumps. (8) Pipette off S.N.F. (9) Centrifugalize once more. (10) Use the S.N.F. pipetted off from below the immediate surface layer.

Notes.—[1] For opsonic index estimation.

A3·223 B. MALLEI.—(1) Use a 72-hr. culture on glycerin agar with a reaction 1·6 per cent acid to phenolphthalein. (2) Sterilize by heating 2 hr. at 60C. (3) Add 0·5 per cent carbolized 0·85 S.S.S. to make a suspension of the killed surface growth. (4) Standardize. (5) Dilute to requirements. (6) Filter rapidly through thin paper. (7) Test purity by staining with Gram and with a counterstain.

A3·224 B. MALLEI.—(1) Make a suspension of a 72-hr. glycerin agar growth with D.W. (2) Sterilize 2 hr. at 60C. (3) Standardize. (4) Dilute to required concentration. (5) Shake along with glass beads 3 hr. on two successive days. (6) Add sufficient sod. chloride crystals to make isotonic. (7) Keep for use[1] in a cool dark place.

Notes.—[1] In agglutination and complement fixation tests.

A3·225 B. MALLEI.—(1) Make a suspension of a 72-hr. glycerin agar growth with strong formalin to sterilize. (2) Centrifugalize the formalin suspension at high speed. (3) Pipette off the S.N.F. (4) Replace the formalin by washing with 1 per cent S.S.S. (5) Make a suspension of the bacterial sediment with 1 per cent S.S.S.

A3·226[1] B. ANTHRACIS.—(1) Subculture the strain used, daily for 10 d. on agar, incubating at 42C. until a sporeless and vigorous growth is obtained. (2) Make a suspension of a 12-hr. growth at 42C. in 0·5 per cent formalinized 0·85 S.S.S. (3) Shake 48 hr. (4) Allow to stand several days. (5) Test for sterility. (6) Shake again 24 hr. (7) Allow to stand overnight. (8) Pour off the S.N.F. and filter through four thicknesses of cheese cloth. (9) Dilute filtrate with 0·5 per cent formalinized

0·85 S.S.S. to give a concentration equivalent to about 1·5 mgm. dried B. typhosus per c.c.

Notes.—[1]For thermo-precipitin test.

A3·227[1] SPIROCHÆTA PALLIDA.—(1) Grind up in a mortar a 24-d. culture of Spirochæta pallida on ascitic agar (**M. 2·72**), culture and all. (2) Add little by little to the paste obtained a culture in ascitic fluid to make a suspension. (3) Sterilize in a water bath 60 min. at 60C. (4) Add liquid pure carbolic acid to 0·5 per cent. (5) Test for sterility by culture and by animal inoculation. (6) Keep in the ice-chest.

Notes.—[1]Luetin for luetin test ; used in dose of 0·5 c.c. intracutaneously with appropriate control.

A3·228.—SPORE BEARING ANAËROBES. (1) Grow 48 hr. in glucose bouillon under anaërobic conditions. (2) Centrifugalize culture at high speed. (3) Wash sediment with 0·85 S.S.S. two or three times. (4) Suspend in 0·85 S.S.S. in concentration about 800 million per c.c. (5) Administer an initial dose of 1 c.c. to a rabbit intravenously for the production of an agglutinating serum.

A3·23 DESICCATED.

A3·231.—(1) Remove the bacterial growth with a platinum loop or scraper from the surface of agar slopes. (2) Spread thinly on sterile watch glasses or Petri dishes. (3) Dry to constant weight *in vacuo* over sulphuric acid or calc. chloride, or in the incubator. (4) Detach the dried antigen from the glass. (5) Sterilize[1] by dry heat at 120C. (6) Preserve in a desiccator. (7) Weigh out for use in a weighed sterile watch glass.[2] (8) Add a measured quantity of 0·85 S.S.S. (9) Allow to soak overnight in a moist chamber.[3] (10) Grind up to give a suspension with a sterile glass rod, in the watch glass. (11) Add 0·85 S.S.S. to give the final concentration required.

Notes.—[1]Sterilization may be dispensed with in many cases. [2]Another method consists in adding the quantity of sod. chloride crystals, at this stage, sufficient to make an isotonic sol. with the D.W. required to give the bacterial concentration desired. The powder is ground up in a mortar with the salt crystals and the D.W. added gradually. [3]A glass receptacle with close fitting cover containing at the bottom blotting paper or wool soaked in 1-1000 mercury perchloride.

A3.232.—(1) Centrifugalize the bacterial suspension in D.W. at high speed (**A3·287 ; A3·312**). (2) Dry the sediment.

A3·233 v. A3·163.

A3·24 TOXIN.

A3·241 B. DIPHTHERIÆ.—(1) Use a suitable strain[1] of organism. (2) Sow[2] daily on bouillon for preservation[3] of stock strain. (3) Use for

production of toxin a glucose[4] bouillon or pepsin digest (**M. 3·921**) of reaction 0·5 per cent acid[5] to phenolphthalein and containing 0·5 per cent calc. carbonate[5] or chips of marble.[6] (4) Sow from a 24-hr. bouillon growth of stock strain on the surface of the toxin medium, in flasks only half filled in order to give a large free surface. (5) Incubate 7 d. (6) Test purity and reaction. (7) Add liquid pure carbolic acid[7] to give a concentration of 0·5 per cent. (8) Shake up. (9) Allow to stand 48 hr. (10) Siphon[8] off the clear S.N.F. (11) Filter through an unglazed porcelain or Berkefeld candle. (12) Keep in a cool dark place in completely filled[9] tightly stoppered bottles until the toxin has become stable.[10]

Notes.—[1]Toxigenic. One cubic centimetre of a bouillon culture should kill a 350 grm. G. P. by subcutaneous injection in 30 hr. The commonly used strain is that known as the Park Williams bacillus. [2]Or sow upon coagulated serum and keep after incubation at a temperature of 10 to 12C. Subculture twice or thrice in bouillon before using as seed. [3]Transfer only a surface growing portion, to maintain the essential surface character of the growth. [4]Best added sterilely from a solution to the remainder of the medium at the time of sowing. [5]If much higher than 0·5 per cent the toxin produced is not likely to be very potent. [6]To neutralize acid formed. [7]Kills the bacilli and assists in the sedimentation. [8]Or filter through filter paper. [9]Or under a layer of toluol. [10]If used before attainment of stability the value of the M. L. D. obtained will not be valid within wide limits for subsequent determinations. Stability may not be attained in from 9 to 12 m.

A3·242 B. DIPHTHERIÆ.—(1) Grow a suitable strain of organism in ordinary peptone bouillon containing 15 per cent blood serum of the animal species to which the toxin is to be administered. (2) Incubate 3 wk. (3) Sterilize by heating in the water bath 1 hr. at 65C. (4) Filter through an unglazed porcelain or Berkefeld candle.

A3·243 B. TETANI.—(1) Use a suitable[1] strain of organism. (2) Use for production of toxin a glucose bouillon of reaction 0·5 per cent acid to phenolphthalein and containing a small quantity of calc. carbonate[2] or chips[2] of marble. (3) Sow with B. tetani. (4) Incubate 10 d. anaërobically under hydrogen. (5) Test purity. (6) Add liquid pure carbolic acid to give a concentration of 0·5 per cent (7) Shake. (8) Allow to stand 48 hr. (9) Filter through an unglazed porcelain or Berkefeld candle. (10) Reduce acidity[3] of filtrate to nearly neutral to phenolphthalein. (11) Test potency of toxin.[4] (12) Keep in a cool dark place in completely filled tightly stoppered bottles.

Notes.—[1]Toxigenic. Subcultured daily in glucose bouillon for 2 wk. before use. [2]To neutralize acid formed. [3]Lessens reaction of injection. [4]Subcutaneous injection of 0·0001 c.c. should kill a 300 grm. G. P. within 5 d.

A3·244 B. BOTULINUS.—(1) Grow anaërobically under vaselin or hydrogen a suitable[1] strain in glucose bouillon[2] containing 0·5 per cent calc. carbonate[3] or chips of marble.[3] (2) Incubate 3 wk. at 20C.

Notes.—[1]Toxigenic. [2]Made—if available—from pig flesh. [3]To neutralize acid formed.

A3·245 V. SEPTIQUE.—(1) Inoculate a G. P. intramuscularly with 0·5 to 1 c.c. of a 24 to 48-hr. broth culture. (2) Remove the liver as soon as possible after death under sterile conditions. (3) Cut up into pieces in a sterile Petri dish. (4) Use as culture medium 0·2 per cent glucose bouillon of reaction about pH7·8. (5) Drop pieces of liver into 100 c.c. of this medium. (6) Incubate.[1] (7) Remove the medium from the incubator when the bacteria agglutinate[2] and have sunk to the bottom. (8) Decant the S.N.F. into centrifuge tubes. (9) Centrifugalize at high speed till the S.N.F. is water-clear. (10) Pour off the S.N.F.=toxin. (11) Test the potency[3] of the toxin intravenously in rabbits. (12) Filter through a Berkefeld candle. (13) Test the filtrate for sterility. (14) Store in the ice-chest in sealed tubes.

Notes.—[1]The anaërobic conditions are supplied by the action of the fresh tissues. [2]About 24 hr. [3]A good toxin will produce death in a 2000 grm. rabbit in 3 to 10 min. in a dose of 0·1 to 0·5 c.c.

A3·3 FUNGI.

A3·31.—(1) Sow small particles ofgrowth[1] in flasks containing maltose peptone water of composition :—chemically pure maltose 4 ; peptone 1 ; D.W.100. (2) Leave with reaction—which is acid—unadjusted. (3) Place on the top of an incubator for 2 wk. (4) Test purity. (5) Filter through sterile moistened filter paper. (6) Wash the growth with 0·85 S.S.S. (7) Transfer to a Petri dish or to platinum foil. (8) Dry *in vacuo* over sulphuric acid or calc. chloride. (9) Weigh. (10) Grind up in a mortar with crystals of sod. chloride. (11) Add sufficient D.W. to make a 0·85 per cent sol. with the sod. chloride. (12) Sterilize (13) Test sterility. (14) Add liquid pure carbolic acid to give a concentration of 0·5 per cent. (15) Place in ice-chest. (16) Shake daily. (17) Allow the heavier particles to settle. (18) Pipette off the S.N.F. and use as vaccine.

Notes.—[1]Organisms such as Achorion schoenleinii, Tinea tonsurans, etc.

A3·32.—(1) Grind up a portion of a 9-wk. culture on Sabouraud's medium (**M. 4·522**) in a mortar with 0·85 S.S.S. to make a suspension. (2) Filter through moistened paper. (3) Examine microscopically to see that the suspension consists of free spores and is of sufficient concentration.

(4) Add liquid pure carbolic acid to give a concentration of 0·5 per cent. (5) Use for serum tests.[1]

Notes.—[1]Agglutination, complement fixation, cuti-reaction, etc., tests.

A3·4 ANIMAL TISSUES AND FLUIDS.

A3·41 BLOOD.

A3·411.[1]—(1) Take up in a sterile 20 c.c. syringe 3 c.c. of 0·85 S.S.S. containing 2 per cent sod. citrate. (2) Aspirate aseptically into the syringe 10 c.c. blood from the jugular vein of the sheep. (3) Empty the contents of the syringe into a large T.T. containing 1 c.c. 2 per cent sod. citrate in 0·85 S.S.S. (4) Mix. (5) Preserve till required. (6) Draw out quantities for use and fill into centrifuge tubes. (7) Fill up the tubes with a relatively large vol. of 0·85 S.S.S. (8) Centrifuge. (9) Remove the S.N.F. down to the corpuscular sediment and fill up with fresh 0·85 S.S.S. (10) Shake up to mix. (11) Centrifuge. (12) Repeat the operation of addition of fresh salt sol. at least three times.[2] (13) Dilute the washed erythrocyte sediment 1 in 20 with 0·85 S.S.S. for use in the Wassermann test.

Notes.—[1]For production of hæmolytic sera and as reagent in hæmolytic titration and in the Wassermann or similar tests. [2]In order effectually to remove all traces of serum.

A3·412.—(1) Obtain sheep's blood in the slaughter house as aseptically[1] as possible. (2) Collect the blood, as the animal is being killed, in a wide mouthed, litre-sized, glass stoppered, sterile bottle containing pieces of glass and iron rod. (3) Fill only half-full. (4) Shake the bottle 5 min. or longer to defibrinate. (5) Preserve as defibrinated blood in the ice-chest at a temperature not exceeding 6C. till required. (6) Draw out quantities for use and fill into centrifuge tubes. (7) Wash in the centrifuge. (8) Dilute the washed erythrocyte sediment 1 in 20 with 0·85 S.S.S. or with 5 per cent pure glucose sol.

Notes.—[1]Allow the first rush of blood to escape before collecting it.

A3·413 SENSITIZED.—(1) Prepare a dilution of the hæmolytic serum which is ten times as strong as that representing its imiting titre. (2) Add it quickly to an equal quantity of 5 per cent suspension of the appropriate erythrocytes. (3) Use[1] in the Wassermann test.

Notes.—[1]For some purposes the erythrocytes after having been in contact with the serum are washed free of it again.

A3·414 BLOOD PRECIPITINOGEN.—(1) Obtain blood of animal for which precipitin is required. (2) Defibrinate. (3) Dry[1] *in vacuo*

over sulphuric acid or calc. chloride. (4) Sterilize the dried blood at 150C. 1 hr.[2] (5) Preserve for use.

Notes.—[1]The blood may be used at once without drying. [2]This treatment does not affect its antigenic property.

A3·415[1] BLOOD RINDERPEST.—(1) Obtain defibrinated sterile blood from an animal at the height of the disease on the fourth day of fever. (2) Make a complete post-mortem examination to exclude concurrent disease.

Notes.—[1]Used by subcutaneous injection in immunization along with specific immune serum.

A3·416 BLOOD ANTHRAX.—(1) Mix the test blood[1] with 6 to 8 vol. 0·85 S.S.S.[1] containing 1-1000 acetic acid. (2) Boil some minutes. (3) Filter when cool to obtain a clear fluid. (4) Add to specific serum to obtain a ring precipitate[2] at the interface of the two fluids.

Notes.—[1]Or a suspension of spleen pulp in 0·85 S.S.S. [2]Thermo-precipitin test.

A3·417[1] BLOOD SERUM v. Blood Serum Collection.

Notes.—[1]Used as antigen in the production of precipitin sera.

A3·418 BLOOD STAIN.—(1) Place the blood stained material in a T.T. with 5 c.c. 0·85 S.S.S. (2) Shake for 15 min. or until the solution presents a lasting[1] foam. (3) Filter[2] or centrifugalize.[2]

Notes.—[1]This is evidence that the protein is in sol. in a concentration of at least 1-1000 : old specimens must often be left in the salt sol. in the ice-chest 24 hr. or longer. [2]To obtain an absolutely clear fluid.

A3·419 BLOOD STAIN.—(1) Place the blood stained material in a T.T. in water and allow to soak. (2) Do not shake.[1]

Notes.—[1]Otherwise a muddy suspension will be obtained instead of a clear one.

A3·42 BODY FLUIDS.[1]

Notes.—[1]Other than blood and blood serum.

A3·421 BILE, RINDERPEST.[1]—(1) Obtain fluid bile, unstained with blood, of animals which die of rinderpest on the fifth or sixth day from the commencement of fever. (2) Make a complete post-mortem examination to exclude concurrent disease. (3) Keep the bile in the dark in an ice-chest, but not for more than 10 d.

Notes.—[1]Used by subcutaneous injection in prophylactic immunization.

A3·422 CEREBRO-SPINAL FLUID.—(1) Centrifuge to clear. (2) Use as antigen in test with specific serum.

A3·423 HYDATID FLUID. (1) Obtain hydatid fluid from a cyst of a sheep, or from a patient. (2) Fill into capsules.[1] (3) Seal.

(4) Sterilize by heating 20 min. at 60C. on each of three successive days. (5) Use as antigen, when required, by precipitin test or by fixation of complement test.

Notes.—[1]The fluid may also be used fresh, and may also conveniently be sterilized by filtration through a porcelain or Berkefeld candle.

A3·43 BRAIN[1] AND SPINAL CORD.[1]

Notes.—[1]Used as antigen in rabies immunization.

A3·431 DESICCATED ANTIGEN[1] RABIES.—(1) Remove the medulla of a fixed virus rabbit which has been killed not earlier than the ninth day after subdural inoculation. (2) Make a suspension of the medulla by triturating in 1 c.c. 0·85 S.S.S. (3) Trephine a rabbit. (4) Inject 0·2 c.c. suspension subdurally. (5) Return the rabbit to its cage. (6) Take an inoculated animal daily, which has shown paralysis and been killed not earlier than the ninth day after inoculation. (7) Expose the spinal cord and medulla with aseptic precautions. (8) Sever the cord just below the medulla. (9) Snip off a portion into nutrient bouillon. (10) Incubate 24 hr. as a test of sterility. (11) Remove the spinal cord intact. (12) Divide it into two equal pieces and suspend each separately by sterile silk threads in a sterile glass jar containing sticks of pot. hydroxide. (13) Place the fourth ventricle and medulla in a sterile dish and use for continuing the passage inoculations in the rabbit. (14) Make a post-mortem examination of the rabbit for disease, which, if present, will necessitate rejection of the material. (15) Keep the jars in which the spinal cords are to undergo desiccation at 22C. (16) Make a suspension of 1 cm. of cord, at the various periods of drying in 3 c.c. of 0·85 S.S.S.[2]

Notes.—[1]Pasteurian method. [2]Should contain 20 per cent glycerin and 0·5 per cent carbolic acid, if the suspension is to be sent to a distance.

A3·432 FRESH ANTIGEN[1] RABIES.—(1) Remove the medulla of a fixed virus rabbit. (2) Weigh. (3) Grind up thoroughly in a sterile mortar with 0·85 S.S.S. to make a 1-100 suspension. (4) Make up a series of dilutions of the suspension with 0·85 S.S.S., 1-10,000; 8,000; 6,000; 5,000; 2,000; 1,000; 500; 200.

Notes.—[1]The antigen used in the method of Hœgyes for rabies immunization.

A3·433 CARBOLIZED ANTIGEN[1] RABIES.—(1) Remove the whole brain and medulla of a fixed virus rabbit. (2) Weigh. (3) Grind up thoroughly in a sterile mortar with 0·85 S.S.S. containing 1 per cent carbolic acid so as to make a 2 per cent suspension. (4) Filter through sterile gauze placed in a fine mesh strainer. (5) Place in incubator 24 hr.

to allow the carbolic acid effectually to kill the rabies virus. (6) Dilute with an equal vol. of 0·85 S.S.S. (7) Place in ice-chest till required for use. (8) Dilute again at time of use with an equal vol. of 0·85 S.S.S.

Notes.—[1]Used in the Pasteur Institutes of India.

A3·44 MEAT, FLESH, MUSCLE.

A3·441.—(1) Pass a portion of flesh or meat, freed as far as possible from fat and fascia, of about 250 grm. weight through a Bunsen flame to sterilize the exterior. (2) Cut through with a sterile knife. (3) Remove about 50 grm. from the centre with sterile precautions. (4) Place in a flask and cover with ether.[1] (5) Leave 18 hr. (6) Pour off the S.N.F. (7) Wash twice with 0·85 S.S.S. (8) Cut up or mince finely. (9) Place in a flask and cover with 100 c.c. 0·85 S.S.S. (10) Leave 6 hr. at R.T. shaking up from time to time to aid the extraction. (11) Test by foam test (**A3·481**) for sufficiency of extraction. (12) Filter through moistened filter paper, or through a Berkefeld candle, or centrifuge at high speed to obtain a clear fluid. (13) Dilute[2] with 0·85 S.S.S. (14) Make reaction, if acid, neutral to litmus paper with sod. hydroxide sol. (15) Carry out the ring precipitin test with the clear fluid obtained.

Notes.—[1]To remove fat. [2]Heat 2 c.c. of the fluid in a T.T. and add a drop of dilute acetic acid. Dilute the fluid with clear 0·85 S.S.S. until this test gives rise to merely a general cloudiness with production of a slight precipitate after 5 min. standing.

A3·442.[1]—(1) Extract the meat under test with ether to remove fat. (2) Cut up or mince finely. (3) Add 1 c.c. 0·85 S.S.S. per gramme of meat. (4) Leave overnight in the ice-chest. (5) Pipette off the S.N.F. (6) Mix with 0·1 c.c. inactivated specific[2] serum and 0·05 c.c. fresh G. P. serum. (7) Carry out fixation of complement test with appropriate controls.

Notes.—[1]*E.g.*, sausage meat. [2]*E.g.*, rabbit anti-horse serum.

A3·45 MUCUS, SPUTUM.[1]

A3·451.—(1) Place about 5 c.c. test sputum in a centrifuge tube. (2) Heat in boiling water in order to coagulate. (3) Break up the coagulum. (4) Add a small[2] quantity of 0·85 S.S.S. (5) Heat again in boiling water, shaking frequently. (6) Centrifugalize. (7) Carry out the 'ring' precipitin test with the clear S.N.F. and specific or 'type' sera.

Notes.—[1]Used chiefly for the recognition of 'type' in pneumococci. [2]Dilute as little as possible.

A3·452.—(1) Add 5 per cent antiformin to sputum to dissolve it. (2) Boil. (3) Over-neutralize while hot with acid to precipitate albumin,

(4) Centrifuge. (5) Pipette off the clear S.N.F. and neutralize it. (6) Add alc. to precipitate the specific antigen. (7) Centrifugalize. (8) Reject the S.N.F. (9) Extract the deposit with as small a quantity as possible of 0·85 S.S.S. (10) Centrifuge. (11) Carry out the 'ring' precipitin test with the clear S.N.F. and specific or 'type' sera.

A3·453.[1]—(1) Make a suspension 1-6 of freshly passed blood stained mucus from a stool. (2) Remove fæcal matter. (3) Boil 5 min. (4) Centrifuge at high speed. (5) Carry out the 'ring' precipitin test[2] with the clear S.N.F. and specific serum.

Notes.—[1]Used for diagnosis of dysentery. [2]Thermo-precipitin test.

A3·46 SNAKE VENOM.

A3·461.—(1) Cut away the lower jaw of the snake with a pair of scissors. (2) Pin the head, in the middle line, to the dissecting board. (3) Draw the poison fangs out of their sheaths. (4) Isolate carefully the two poison ducts. (5) Ligature with thread. (6) Dissect out the glands and ducts entire. (7) Sever the duct between ligature and gland. (8) Squeeze[1] each gland towards the cut ends of the ducts. (9) Collect the liquid obtained. (10) Dry *in vacuo* over sulphuric acid or calc. chloride. (11) Detach the dried scales of venom. (12) Place in a T.T. (13) Seal in the blow pipe flame. (14) Keep in a cool dark place.

Notes.—[1]This procedure may be followed with the glands *in situ* and the poison collected as it issues from the duct openings. Another method is to grind up the glands in a mortar, add glycerin and strain through cloth.

A3·462.—(1) Tip the living snake out on the floor. (2) Place a stick held in the left hand on the neck of the animal as it moves along. (3) Place the right foot on the body of the snake. (4) Seize the snake in the right hand as close up to the head as possible and grip the body of the snake with the released left hand. (5) Drop the snake rapidly into a suitable glass vessel containing wool saturated with chloroform. (6) Slip a cover without any delay over the mouth of the vessel. (7) Leave the snake till dead. (8) Place a Petri dish between the upper and lower jaw with the raised fangs projecting into the dish. (9) Squeeze the two glands towards the duct openings to eject the venom into the dish. (10) Dry *in vacuo* under sulphuric acid or calc. chloride.

A3·463.—(1) Fix the head of the snake with a stick. (2) Seize it with snake forceps and grasp it with the left hand as close as possible to the head. (3) Grasp the body of the snake with the other hand. (4) Have an assistant squeeze the two glands and collect the poison as it issues from the openings of the salivary ducts.

A3·5 PLANT TISSUES AND FLUIDS.

A3·51 ABRIN.[1]—(1) Grind up the seeds in a mortar. (2) Treat with ether and then with alc. to remove fats, lecithin, cholesterin, alkaloids, etc. (3) Treat with 10 per cent sod. chloride at 37 to 40C. 24 hr. (4) Filter. (5) Saturate the filtrate with ammon. sulphate. (6) Collect and dry the precipitate.[2] (7) Digest 0·35 grm. of the powder at 37C. with a potent pepsin hydrochloric acid mixture. (8) Neutralize. (9) Precipitate by 60 per cent saturation with ammon. sulphate. (10) Wash the precipitate with similar ammon. sulphate sol. (11) Dissolve the precipitate in 90 c.c. sod. chloride sol.

Notes.—[1] A plant toxin obtained from the seeds of jequirity (Abrus precatorius). A similar procedure is used for the preparation of ricin and crotin. [2] This is the preparation ordinarily called abrin. If the sod. chloride and ammon. sulphate are to be removed from the preparation, it must be subjected to dialysis. The further procedure given is to effect greater purification.

A3·52 POLLEN.

A3·521.—(1) Cut the heads of Timothy grass[1] at the time the pollen receptacles are ready to shed their pollen grains. (2) Place in water over a large sheet of white paper. (3) Leave in a quiet place protected from draughts of air. (4) Gather the fallen pollen by brushing together the grains with a camels' hair brush. (5) Weigh. (6) Grind up in a mortar with sand or broken glass. (7) Add D.W. to give a 2 per cent suspension. (8) Assist the extraction by alternately freezing and thawing. (9) Filter through paper. (10) Adopt as a unit the quantity of extract obtained from 1-1000th mgm. pollen. (11) Express the strength of the extract in units per c.c. (12) Test the strength of the extract by instilling one drop of 4-unit strength into the conjunctival sac of the person to be treated. (13) Increase the unit strength until a reaction is obtained.[2] (14) Base the treatment on this strength.[3]

Notes.—[1] Phleum pratense. Other pollens or a mixture may be used. In America the following pollens are utilized :—Timothy, red top, June and orchard grass; rye, sorrel dock, daisy, maize, ragweed and golden rod. [2] The *minimum reacting dose* in the Hay Fever ophthalmic test. [3] A suitable initial dose is 0·3 c.c. of that dilution of pollen toxin which gives the ophthalmo-reaction.

A3·522.—(1) Procure suitable pollen. (2) Grind up in a mortar with sand or broken glass. (3) Add 0·85 S.S.S. containing 0·5 per cent carbolic acid. (4) Place 10 hr. in incubator. (5) Centrifugalize[1] at high speed and use the S.N.F. as antigen in the ophthalmic test for Hay Fever, or the precipitate obtained from the S.N.F. with 9 vol. 95

per cent alc., with acetone, or by salting out with mag. sulphate or ammon. sulphate.

Notes.—[1] The extract may also be cleared by filtration through an unglazed porcelain or Berkefeld candle.

A3·6 WASSERMANN TEST.

A3·61 EXTRACT.[1]

Notes.—[1] The antigen or lipoid extract used in the Wassermann serum test.

A3·611.—(1) Cut up finely the liver of a syphilitic fœtus. (2) Weigh. (3) Add 4 c.c. per grm. 0·5 per cent carbolized 0·85 S.S.S. (4) Leave to extract[1] 24 hr. with frequent shaking. (5) Centrifuge. (6) Pipette off the slightly opalescent S.N.F. for use. (7) Standardize.

Notes.—[1] The original Wassermann antigen. Almost entirely replaced now by alc. extracts.

A3·612.—(1) Select fat-free portions of heart or liver of either man, ox, dog, rabbit, or G.P. (2) Cut up finely. (3) Weigh. (4) Grind up with clear sand or broken glass in a mortar and add 10 c.c. abs. alc. per grm. (5) Decant the suspension obtained into a well-stoppered glass bottle. (6) Place in incubator 7 d. shaking at frequent intervals. (7) Filter through two thicknesses of paper which has been washed through with ether and allowed to dry. (8) Siphon off the S.N.F. (9) Keep[1] in ice-chest. (10) Prepare fresh for the test :—S.N.F. 3 ; 1 per cent cholesterin in abs. alc. 2. (11) Dilute this mixture rapidly 1 in 15 by adding to it the total quantity of 0·85 S.S.S. required.

Notes.—[1] Will keep 6 m.

A3·613.—(1) Select a fat-free portion of rabbit heart. (2) Cut up finely. (3) Weigh. (4) Grind up with clean sand or broken glass in a mortar. (5) Add 5 c.c. 95 per cent alc. for every grm. of heart to make a suspension. (6) Heat 2 hr. at 60C. in a water bath. (7) Filter through moistened paper. (8) Keep the filtrate as stock antigen. (9) Dilute fresh for use 1-10 with 0·85 S.S.S.

A3·614 ACETONE INSOLUBLE.—(1) Select fat-free portions of either human, ox, dog, rabbit or G. P. heart or liver. (2) Cut up finely. (3) Weigh. (4) Grind up with clean sand or glass in a mortar and add 10 c.c. abs. alc. per grm. (5) Boil 15 min. in a water bath.[1] (6) Filter through moistened paper while hot. (7) Evaporate the filtrate over boiling water. (8) Mix the sticky residue obtained with ether, 1 c.c. for each grm. of substance originally used. (9) Place overnight in the ice-chest. (10) Centrifugalize at high speed, and obtain a perfectly clear S.N.F. (11) Add the S.N.F. to 10 vol. chemically pure acetone. (12) Allow

the precipitate to settle. (13) Reject the supernatant acetone. (14) Centrifugalize the sedimented precipitate. (15) Pipette off the remaining supernatant acetone. (16) Add to the sediment 10 vol. chemically pure methyl alc. (17) Stir well to bring about sol. (18) Centrifugalize. (19) Keep the S.N.F. as stock antigen. (20) Test for antigenic, anticomplementary, and hæmolytic properties. (21) Dilute fresh for use 1-10 with 0·85 S.S.S.

Notes.—[1]Or place in incubator in a well-stoppered glass bottle 7 d. shaking at frequent intervals.

A3·615 ACETONE INSOLUBLE.—(1) Select a fat-free portion of calf heart. (2) Cut up finely, taking care that the tissue fluid is not lost in the process. (3) Weigh. (4) Place in a well-stoppered glass bottle. (5) Add[1] 1·25 c.c. 95 per cent alc. per grm. (6) Shake. (7) Leave 3 d. at R.T. (8) Filter through moistened filter paper. (9) Dry the residue left on the filter 24 hr. in the incubator. (10) Place the powder obtained in a well-stoppered glass bottle. (11) Add chemically pure acetone, 2 c.c. per grm. of original tissue. (12) Keep 7 d. at 20C. (13) Replace the acetone with fresh acetone. (14) Leave 24 hr. (15) Filter through paper. (16) Dry the residue left on the filter paper in the incubator. (17) Place the powder obtained in a well-stoppered glass bottle. (18) Add 2 c.c. 95 per cent alc. per grm. of original tissue. (19) Keep 9 d. at 20C. (20) Filter. (21) Keep[2] the filtrate as stock antigen. (22) Place, when required for use, 0·5 c.c. in a large watch glass. (23) Evaporate to a residue in the incubator. (24) Add 2 c.c. D.W. drop by drop to the residue to bring it into suspension. (25) Add the suspension to 18 c.c. 0·85 S.S.S. (26) Shake to mix. (27) Use.

Notes.—[1]This first addition of alc. is intended to coagulate albuminoids without removing lipoids. In this proportion and with the dilution of the tissue juice it does not dissolve the lipoids. [2]Keeps indefinitely.

A3·62 ERYTHROCYTE SUSPENSION.

A3·621.—(1) Use washed sheep erythrocytes (**A3·411, A3·412**) or sensitized erythrocytes (**A3·413**) in 5 per cent suspension in 0·85 S.S.S.

A3·622.—(1) Prepare: Washed[1] deposit of sheep's erythrocytes 5; 1-10 formalin 0·1. (2) Keep[2] as stock mixture. (3) Prepare[3] at time of use:—formalinized erythrocytes 6; 0·85 S.S.S. 100.

Notes.—[1]The blood is obtained from the slaughter house. It is caught as aseptically as possible in sterile bottles containing 1-5th of their vol. of 5 per cent citrated 0·85 S.S.S. As soon as possible after collection the erythrocytes are washed. The final deposit is packed as firmly as possible by prolonged centrifuging. [2]These formalinized cells keep perfectly well for at least a week. [3]Use a 1 c.c pipette previously wetted

with 0·85 S.S.S. to measure the formalinized erythrocytes. This 6 per cent suspension is sensitized for use in the Wassermann test by mixing with an equal quantity of an appropriate dilution of the hæmolytic serum, or the mixture may be made up directly from the formalinized erythrocytes as follows :—formalinized erythrocytes 0·25 ; 0·85 S.S.S. 3·9 ; diluted hæmolytic serum 4·1. The dilution of hæmolytic serum used is the greatest which gives by titration maximum sensitization of erythrocytes.

A3·623.—(1) Collect sheep blood as sterilely as possible. (2) Defibrinate. (3) Prepare :—formalin 1 ; defibrinated blood 800. (4) Keep in the ice-chest undisturbed[1] till required for use. (5) Wash at time of use three times with 0·85 S.S.S. (6) Use the washed deposit in 5 per cent suspension in 0·85 S.S.S.

Notes.—[1] Will keep thus for 4 wk. without hæmolysis.

A3·624.—(1) Collect one part sheep blood in four parts Locke's citrate gelatin sol.[1] (2) Wash the erythrocytes twice with the same sol. (3) Preserve till required in Locke's sol.[1] to which sufficient isotonic saccharose sol.[2] has been added to give a concentration of 2·8 per cent saccharose. (4) Draw off sufficient suspension for use in test as required.

Notes.—[1] Locke's sol. has the composition :—sod. chloride 9·2 ; sod. bicarbonate 0·05 ; pot. chloride 0·1 ; calc. chloride 0·1 ; water 1000. The addition of 1 per cent sod. citrate and 1-8th per cent gelatin makes of it Locke's citrate gelatin sol. [2] 10·3 per cent.

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B. FRIEDLÆNDER.—A3·1502.
FUNGI.—ANTIGEN A3·3.

D. GONORRHŒA.—ANTIGEN DETOXICATED A3·1502.—ANTIGEN SERUM REACTIONS 3·2111.—SENSITIZED VACCINE A3·1421.

HÆMOLYTIC.—SERUM ANTIGEN A3·4111.
HAY FEVER.—ANTIGEN A3·52.—OPHTHALMIC TEST ANTIGEN A3·52.
HŒGYES.—RABIES ANTIGEN A3·4321.
HYDATID FLUID.—ANTIGEN A3·423.
HYDROPHOBIA.—v. RABIES.

B. INFLUENZÆ.—ANTIGEN DETOXICATED A3·1502.—VACCINE A3·1351.
INTRACUTANEOUS TEST.—ANTIGEN A3·3201.—LUETIN ANTIGEN A3·2271.
ISOTONIC.—SACCHAROSE A3·6242.

JEQUIRITY SEEDS.—ANTIGEN A3·5101.

LIPO-VACCINE.—A3·16.
LIPOID.—EXTRACT WASSERMANN TEST A3·6101.
LOCKE.—CITRATE GELATIN SOLUTION A3·6241.—SOLUTION A3·6241.
LUETIN TEST.—ANTIGEN A3·2271.

B. MALLEI.—AGGLUTINATION TEST A3·2241.—ANTIGEN SERUM REACTIONS A3·223 TO 3·225—BACILLARY EMULSION A3·172.—COMPLEMENT FIXATION A3·2241.—EXTRACT A3·194 A3·195.—VACCINE A3·172.

MALLEIN.—BACILLARY EMULSION A3·172.—EXTRACT A3·194, A3·195.
MEAT.—ANTIGEN A3·44.
MINIMUM REACTING DOSE.—ANTIGEN A3·5212.
MUCUS.—ANTIGEN A3·45.—THERMO-PRECIPITIN TEST A3·451.
MUSCLE.—ANTIGEN A3·44.

NOGUCHI.—ANTIGEN A3·614.

OPSONIC INDEX.—B. TUBERCULOSIS ANTIGEN A3·2221.
OPHTHALMO REACTION.—POLLEN ANTIGEN A3·52.

SP. PALLIDA.—ANTIGEN A3·227.
PASTEUR.—RABIES ANTIGEN A3·4311.
B. PESTIS.—VACCINE A3·1361.
PLAGUE.—v. B. PESTIS.
PNEUMOCOCCUS.—v. D. PNEUMONIÆ.
D. PNEUMONIÆ. ANTIGEN DETOXICATED A3·1502.—TYPE PRECIPITIN TEST A3·4571.
POLLEN.—ANTIGEN A3·52. COLLECTION A3·521.—UNIT ANTIGEN A3·521.
PRECIPITIN TEST.—ANTHRAX A3·4162.—BLOOD ANTIGEN A3·414. A3·4162.—BLOOD STAIN A3·418, A3·419.—MEAT ANTIGEN A3·44.—MUCUS ANTIGEN A3·45.—PNEUMOCOCCUS A3·45.—SAUSAGE MEAT A3·4421.—SERUM ANTIGEN A3·417.—SPUTUM ANTIGEN A3·45.
PRECIPITINOGEN. -v. PRECIPITIN TEST BLOOD A3·414.—SERUM A3·417.
ST. PYOGENES. DETOXICATED A3·1502.—SENSITIZED VACCINE A3·1421.
S. PYOGENES AUREUS.—DETOXICATED A3·1502.—SENSITIZED VACCINE A3·1421.

RABIES.—ANTIGEN A3·431 TO A3·433.—ANTIGEN HŒGYES A3·4321.—ANTIGEN INDIAN A3·4331.—ANTIGEN PASTEUR A3·4311.
RED BLOOD CORPUSCLE.—v. ERYTHROCYTE.
RICIN.—ANTIGEN A3·5101.
RINDERPEST.—BILE ANTIGEN A3·421.—BLOOD ANTIGEN A3·415.

SAUSAGE MEAT. ANTIGEN A3·4421.—COMPLEMENT FIXATION TEST A3·4421.
SENSITIZED.—VACCINE A3·14.—ERYTHROCYTES A3·413.
V. SEPTIQUE.—TOXIN A3·245.
SERUM.—v. BLOOD SERUM.
SNAKE VENOM.—ANTIGEN A3·46.—EXTRACTION A3·46.
SPUTUM.—ANTIGEN A3·45.—PNEUMOCOCCUS TYPE A3·4511.—THERMO-PRECIPITIN TEST A3·451.
STAPHYLOCOCCUS. DETOXICATED A3·1502.—SENSITIZED VACCINE A3·1421.
STREPTOCOCCUS. SENSITIZED VACCINE A3·1421.—DETOXICATED A3·1502.
B. TETANI.—TOXIN A3·243.
THERMO-PRECIPITIN TEST. ANTIGEN A3·2261, A3·4162.—MEAT ANTIGEN A3·441. MUCUS ANTIGEN A3·4532.—SPUTUM ANTIGEN A3·451, A3·452.
TIMOTHY GRASS.—POLLEN A3·521.
TINEA TONSURANS. ANTIGEN A3·3101.
TOXIN. B. BOTULINUS A3·244. B. DIPHTHERIÆ A3·241, A3·242.—B. TETANI A3·243.—V. SEPTIQUE A3·245.
TUBERCULIN. BACILLARY EMULSION A3·171.—OLD A3·191 TO A3·193.

B. TUBERCULOSIS.—ANTIGEN SERUM REACTIONS A3·2111, A3·222.—BACILLARY EMULSION A3·171.—EXTRACT A3·191 TO A3·193.—OPSONIC INDEX TEST A3·2221.—VACCINE A3·171.

B. TYPHOSUS.—DETOXICATED A3·1502.

VACCINE.—AUTOGENOUS A3·12.—BACTERIA A3·11 TO A3·17.—BACTERIAL EXTRACT A3·18.—BOTTLES A3·1333.—DETOXICATED A3·16.—DILUTION A3·1314.—FUNGI A3·31.—LARGE SCALE PRODUCTION A3·131 TO A3·136.—LIPO A3·16.—B. MALLEI A3·172.—SENSITIZED A3·14.—STERILIZATION A3·1334.—STOCK A3·11.—STRENGTH A3·1104.—B. TUBERCULOSIS A3·171.

VIBRION SEPTIQUE.—v. V. SEPTIQUE.

WASSERMANN TEST.—ACETONE INSOLUBLE ANTIGEN A3·614, A3·615.—ANTIGEN A3·6.—ERYTHROCYTE SUSPENSION A3·411 TO A3·413, A3·62.—EXTRACT A3·61.—FORMALINIZED ERYTHROCYTES A3·622, A3·623.—LIPOID EXTRACT A3·6101.—NOGUCHI ANTIGEN A3·614.—ORIGINAL ANTIGEN A3·6111.

A SURVEY OF THE CULICIDÆ OF A RUBBER ESTATE.

BY

R. A. SENIOR-WHITE, F.E.S.

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DURING a period of one year, from March, 1919 to February, 1920, I have had under observation the breeding places of mosquitos on Suduganga Estate, Matale District, Central Province, Ceylon, the results of which are incorporated in this paper.

The planting district of Matale consists of a valley enclosed between the north-eastern and north-western blocks of the main mountain mass of the Island,[1] the distance between the summits of the two ranges being about ten miles. The valley thus enclosed contains a series of ridges, longitudinal earth-folds lying parallel to the main ranges some of the summits of which are raised above the floor of the valley by as much as 1,200 ft. The floor of the valley, which is drained by the Suduganga river, is slightly over 1,000 ft. above sea-level,—the highest summits on the enclosing ranges exceeding 4,000 ft.

Suduganga Estate stretches across both sides of one of these folds—the western boundary being the river, a perrennial stream here flowing between low banks in a sandy bed, liable to sudden floods during periods of heavy rainfall during both monsoons. The annual rainfall varies from 60 to 100 inches.

Though the elevation of the river-bed here is only slightly over 1,000 ft., the highest point on the ridge running through the Estate reaches 1,628 ft., the rise being accomplished in a direct distance only slightly exceeding a quarter of a mile. A similar ridge rises steeply from the western bank.

The estate is planted throughout in para rubber (*Hevea brasiliensis*) which affords a 'cover' comparable with forest,—but the vegetation is of one 'storey' only, the ground between the trees being kept clean weeded. In the months of February and March the trees 'winter,' and all are simultaneously leafless for a few days at this time,—but the new foliage buds during the fall of the old, and the cover is almost immediately renewed. For some weeks afterwards the ground, and all bodies of water, is thickly covered with fallen leaves, which afford excellent cover and food to mosquito larvæ, and it is during these months that those species breeding in permanent bodies of water attain their maximum numbers.

As on the eastern side of the ridge no water is anywhere found, the bungalows, coolie lines and factories have perforce been located on the western slope. The water supply was formerly obtained from wells sunk in various spots, and was poor, with consequent ill health, but four years ago this arrangement gave place to a pumping system, water being taken from a well sunk close to the river and pumped to the highest line site, whence, passing through a 'Jewel' filter and covered storage tank, it is piped down to the various lines and bungalows.

On the plan (Fig. 1.), all points mentioned in this paper are shown. The chain line to the east indicates the summit line of the ridge. To the south this descends nearly to the river-level, thence rises to the trigonometrical station on the heighest point, and passing northwards descends nearly to river-level where the Government road from Matale to Rattota passes through a gap in the ridge. Buildings are shown shaded if inhabited, in outline only if factories, stores, etc. The lines are numbered to correspond with the groups in the malaria figures in Table V.

Down the slope of the ridge are several ravines—shown shaded if they normally contain a small stream, dotted if they only carry flood water.

Mosquito breeding spots are lettered to correspond with Table I.

Collections of water, wells and tanks are indicated in several places which do not carry the letter reference to a breeding place. These may be briefly mentioned.

Factory tank.—A cement tank of large capacity to which water is pumped for manufacturing purposes. It is liable to violent fluctuations of level according to the demands made upon it, though, except for cleaning, it is never absolutely empty. I have never found larvæ in it.

ROAD
STREAM G
PADDY FIELD
H
WELL
8
1051
SWAMP E
WELL D
F
7
BUNGALOW.
1386
WELL
6
TANK
5
1469
RAVINE C
CATTLE
WELL
4
CATTLE
WELL
BUMPS
WELL
1
2
3
BUNGALOW
TANK
FACTORY
1114
SUDUGANGA
1628
SPRING
B
SPRING
1395
1180
STREAM A
ROCKS
1" = 8 CH. (APPROX)

FIG I.

River-bed well.—This was not sampled, owing to depth. I do not think it harbours larvæ, as more favourable breeding places are everywhere available. The suction of the pump intakes cannot render it a suitable breeding place.

Wells between lines 3 *and* 4 *and line* 6 *and bungalow.*—These are old wells not in use since the installation of the water-supply system. They are deep, overgrown with ferns and other plants internally and partially fallen in. They have not been sampled.

Tank at line 6.—This is the head tank of the water-supply system. It is of cement, and of large capacity, exposed to full sunlight. There are violent fluctuations of level as water is withdrawn to the filter. It is regularly cleaned out, and I have never found larvæ in it.

Turning now to the lettered breeding places shown in fig. 1 and in Plates XXIV to XXVI. These were sampled in the middle of each month with a dipper, and the catch bred out. Where a breeding place consists of more than one type, *e.g.*, a stream partly in full sunlight and partly in shade, it was sampled in each kind of location.

Stream A. (Plate XXIV).—This is a shallow-banked ravine always containing a trickle of slowly flowing water, arising amid a tumbled mass of rocks on the hillside. It is liable to sudden freshets during rain, water entering from the contour drains leading into it, and then gets flushed out. The banks are covered with *Mikania scandens* and other creepers, the muddy bed contains water weeds. The *Culicid* fauna is a very restricted one. *Anopheles maculatus* alone breeds in it steadily, occurring in ten months of the year. *Uranotœnia campestris* occurs during March and September in small pools a few inches deep where the stream passes over flat rocks at a point where the morning sun strikes the water. In February, the driest month of the year, when the stream is reduced to a series of puddles, *A. funestus v. listoni* and *Lophoceratomyia minutissima* make their appearance in small numbers, but disappear again after the March rains increase the flow. In December a solitary *Stegomyia scutellaris* was bred from the sample, emerging 12 days after this reached the laboratory. As only one specimen was bred, it is unlikely that the sample was contaminated after taking, but a stream is an unusual breeding place for this household mosquito.

In September my collector discovered a pool among the rocks near the source of the stream, previously overlooked. It was not connected with the stream, and must have been rain-filled, though obviously of a

permanent nature, being about 3 ft. deep, and well shaded. It was full of vegetable debris, black and filthy. A sample produced the heaviest crop of mosquitos throughout the investigation, the species concerned being *Stegomyia scutellaris*, *S. sugens*, *S. trilineata*, and *Ochlerotatus gubernatoris*. There were also several larvæ of an *Odontomyia* *(*Stratiomyidæ*) feeding on fallen leaves in the water. The pool was filled in too big a source of nuisance to be allowed to remain, and was obviously with stones and earth after sampling.

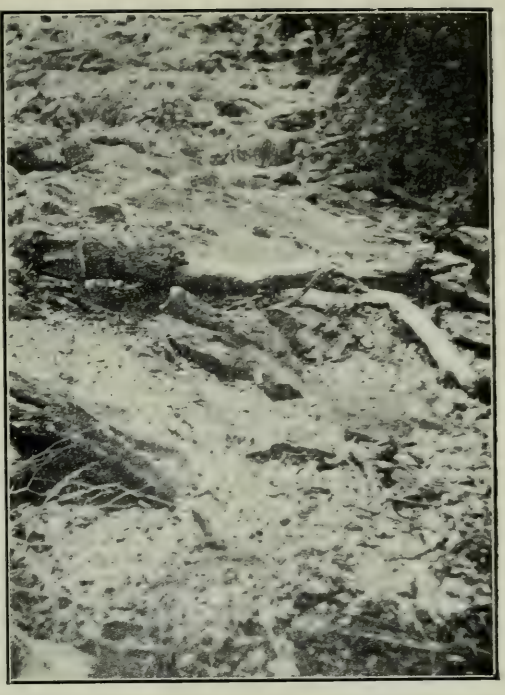
Springs B (Plate XXIV).—These are two small spring-fed pools. The upper is not more than 2 inches deep and 18 inches long, at the foot of a wall of rock just below the ridge, through which water percolates steadily. The lower is a more substantial pool, holding about 4 gallons, among rocks. Both are in deep shade. Neither pool contained larvæ during every month. The upper pool, which contains no water weeds, has a more restricted fauna than the lower. *Anopheles maculatus* appeared with some regularity, being absent in September, and in company with *A. karwari* in November. In October no *Anophelines* were found, the fauna consisting of *Lophoceratomyia minutissima* and *Armigeres sp.* *Uranotænia campestris* bred here in June.

The lower pool, unless disturbed by cleaning out and oiling, proved a fairly steady source of *A. maculatus*, with *A. culicifacies* in March in addition. After oiling in April it yielded *Stegomyia sugens* in May, was again oiled and proved sterile in June, yielding *S. scutellaris* and *Culiciomyia pallidothorax* in July. In August it was again sterile, but by September *A. maculatus* had again found conditions suitable for breeding. In October the very rare *Lophoceratomyia mammilifer* was found, after which it was cleaned out and no larvæ were found until January, in which month and in February *A. maculatus* was again breeding.

Ravine C (Plate XXV).—The head of this ravine consists of a fanshaped block of 'slab rock' through cracks in which springs arise. The largest of these is impounded in a shallow well from which a pipe is laid for coolies to bathe. (Considerations of cost forbid their bathing in the pumped filter-water.) The water from these springs, flowing into the ravine, is absorbed, and its lower course carries flood-water only. The head of the ravine and the water in the well are in full sunlight owing to the rocky outcrop inhibiting vegetation. Taking first the well, the water, except for a few days after periodical cleanings,

* *O. cyanea. Brun.*

STREAM A.—*Mikania scandens* growing on bank. The river in the middle distance.



ROCKS AT HEAD OF STREAM A.—The dark patch of earth in right foreground shows the rock pool after filling in.

UPPER SPRING B.—The rocks with film of water collecting at their foot.

LOWER SPRING B.—In hole beneath boulder and tree trunk.

is foul smelling, and contains much green weed. The depth remains steadily at about 3 feet, although water is always running out through the bathing pipe. Practically only two species breed here, *Anopheles maculatus* in almost every month, and *Culex bitæniorhynchus* when the water is particularly dirty. In January, *C. mimeticus* and *Culiciomyia fusca* are found sparingly. After examination in September, it was treated with potassium permanganate, which rendered it sterile for two months.

The ravine itself, with the small spring pools in the rocks leading out to it, carries *A. maculatus* continuously except in December, in which month the larvæ are probably carried away by flood-water. In January to April *Culex mimeticus* breeds in considerable numbers. In November and February a few *Lophoceratomyia minutissima* are found, the drought of the latter month, reducing the flow to almost *nil*, producing also *Culiciomyia viridiventer*, and a single specimen of the rare *Lophoceratomyia uniformis*.

Spring well D (Plate XXV).—This is another spring impounded for a coolie bathing place, the water, about 5 feet deep and rising to near the surface, flowing out continuously by a pipe inserted into the lower side of the well, which is under shade. It breeds *Anophelines* almost entirely. *A. maculatus* breeds more or less continuously *A. culicifacies* in March, *A. funestus v. listoni* in March, August and September. *A. rossii* occurs in small numbers in January, during which month, as also in February, *Culiciomyia fusca* is found, being the only *Culicine* breeding here.

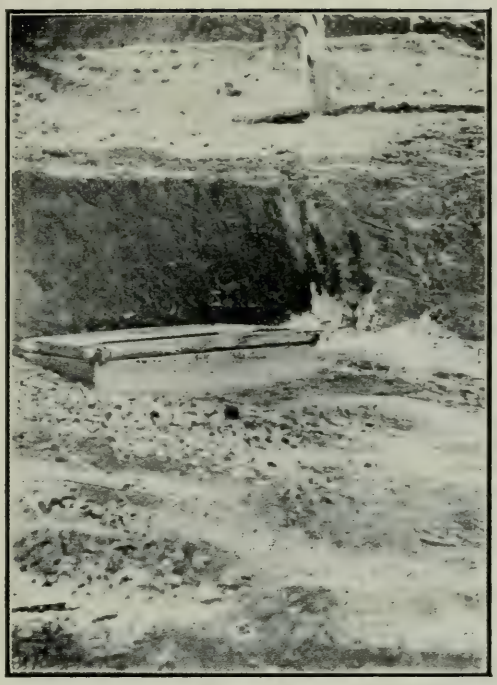
The continued presence of *Anophelines* in this well depends on leaves, etc., floating on the water. The habit of *Anophelines* of backing to the water's edge until the caudal setæ are in contact therewith renders them victims to another inhabitant of this well—a Planarian worm—which kills them in large numbers. This worm cannot swim, but moves along the cement walls of the well just below water, seizing its victims whilst they rest tail to wall. It is seldom found on leaves floating away from the sides, but such serve *Anophelines* equally for the fulfilment of thigmotropism, and in consequence they can be taken in large numbers around them. Specimens of this worm were sent to the Indian Museum, but Dr. Gravely informs me that it cannot be identified there. Present in the first half of the survey period in small numbers, it increased prodigiously from September onwards, apparently clearing the pool of all larvæ during November and December, whilst

January revealed a different fauna. Culicines, not being subject to this thigmotropic reaction, do not fall victims to the worm.

Swamp E (Plate XXV).—Below the above-described well further hillside springs emerge just above the river—making a small area a mass of swampy mud in which footprints and other depressions are instanty converted into miniature pools. This area is exposed to the sun, as attempts to drain it have failed, and no trees grow. It is weeded, however, like the remainder of the estate. The water usually carries an irridescent scum (l ferrous). *A. maculatus* breeds steadily, though the invasion of the swamp by the swarming Planarian from the well in October rendered it sterile in that month, as it was also found in January. *A. rossii* bred here in March, April and June. In May, following on oiling in April, no larvæ were found. In August the water was in a very foul state, and *Culex bitæniorhyncus* appeared. In the breeding bowls the water rapidly became too 'rotten' for even that filth-loving species, and most of the larvæ died. In December *Culiciomyia pallidothorax* appeared in small numbers, whilst in the February drought the swamp afforded a breeding place for *Lophoceratomyia minutissima*, *Culex mimeticus* and *Culiciomyia fusca* to carry through.

River F (Plates XXV & XXVI).—As will be seen from Table I, this only carries mosquitos during a few months of the year, during other months the volume of the current being too great. In the south-west monsoon (April-September), at periods of low water, a breeding place formed, consisting of a backwater amid shifting sandbanks close to the above-described swamp. It was in full sunlight. After the onset of the north-east monsoon a pool, deeply shaded, formed under the bank to the south, midway between stream A and the factory. The water in this pool was cut off from the river when the latter was not in flood. The fauna of these two areas proved, as might be expected, very different. The sunlit backwater, shallow and clean, of the south-west monsoon, swarmed with *Anophelines*. In March *A. culicifacies* alone bred, in April *A. rossii*, *A. jamesii* and *A. sinensis* occurred in company with *Culex bitæniorhyncus*, *C. tritæniorhyncus* and *C. gelidus*, the latter represented, however, by but a single specimen. It is surprising to find *C. bitæniorhyncus* in such a clean situation. Certainly during April the water was covered with fallen leaves, but it is one of the few occasions in which I have bred the species in comparatively clean water. In August the same spot, having been under flood for three months, was again exposed, and *A. funestus v. listoni* bred in it in large numbers.

RAVINE C.—Showing spring walled round to form well and pipe to bathing place.



SPRING WELL D.

SWAMP E.—Sunlight reflected from puddles in the mud.

N.-E. Monsoon pool F in river.

The shady isolated pool, containing flood debris and far from clean, formed in the north-east monsoon, did not harbour malaria carriers. A few *A. rossii* bred in December, with *Lophoceratomyia minutissima* and *Culex mimeticus*, and a single doubtful, damaged specimen of *Stegomyia scutellaris* was reared. In January larvæ failed to discover themselves, but in February the pool was still the breeding ground of *L. minutissima* and *S. scutellaris*.

It is interesting to note that on no occasion was a single specimen of *A. maculatus* bred from either river site, this being the only permanent breeding place examined that did not harbour the larvæ on any occasion.

Stream G (Plate XXVI).—This is a genuine stream containing always considerably greater volume of water than either of those heretofore described. It flows through the estate from a paddy field to the river, the source being a permanent spring of large capacity at the upper end of the paddy field, outside the estate. Its course is in deep, soft soil, into which it has cut its bed to a depth of about 8 feet, with precipitous banks only a few feet apart. Hence it is in deep shadow, save in the lowest portion of its course, below the estate outlet road, where it traverses some flat rocks into which it has been unable to cut. The tributary stream entering it on the south midway is of a similar nature, but with vegetation along its bottom, being less heavily shaded. It is, however, practically dry except in the three months following the north-east monsoon, after which it only carries flood-water.

The stream separates into two distinct sections, clean and foul. From the paddy field to a little below the junction of the tributary, and including the latter, the water is clean and clear, below this point clear, but usually foul, readily 'rotting' on transfer to the breeding bowls. The reason for this is that, in spite of attempts to put a stop to the practice, passers-by on the public road nearby defoecate on rocks in the bed of the stream, and perform their ablutions therein, whilst cattle are also washed in it. The well shown in figure 1 by the Estate outlet road is in use by persons not belonging to the Estate. It is deep, and has not been sampled.

Taking first the upper portion of the stream, *Anopheles maculatus*, *A. funestus v. listoni* and *A. jamesii* are found in March, the latter in the tributary stream only. Until the following February no *Anophelines* are found, but in the latter month a solitary specimen of *A. minimus v. aconitus* was bred. Of *Culicines*, *Culex tritæniorbyncus* and *Culiciomyia fusca* are found in March, the former also in May, but

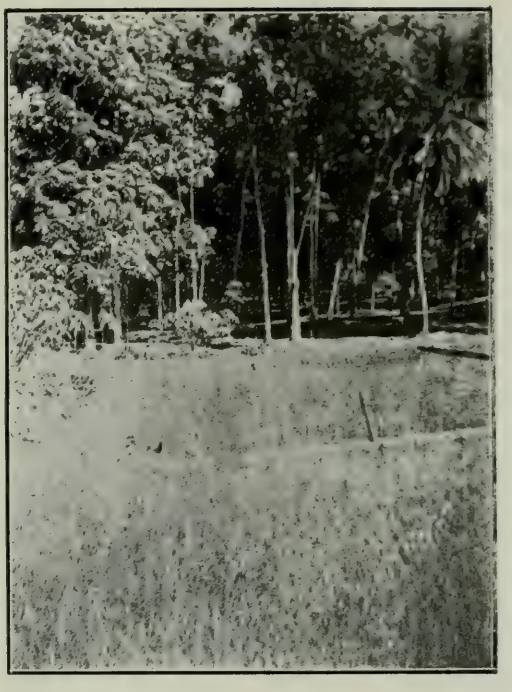
both in small numbers. In the February drought *Lophoceratomyia minutissima and Culex mimeticus* carry through in it, and *Uranotænia campestris* breeds. In the intervening eight months, the samples produced no larvæ. Their presence here seems to be dependent upon that of rubber leaves accumulated in angles of the stream.

The lower portion of the stream bred *A. maculatus* in large numbers in January to May, and August and September. In June *Culex mimeticus* appeared here and in no other place, being accompanied by *U. campestris*. Both again appeared in February. *Lophoceratomyia minutissima* bred in March, July and January, in which latter month certain species appeared that were not found during the remainder of the year in this location. These are *A. rossii, Culiciomyia fusca* and *C. pallidothorax*, the first and last in considerable numbers. A single specimen of *Culex halifaxii* was also bred. This species, I think, is predaceous, like its near relative *C. concolor*. As might be expected, *C. bitæniorhyncus* was often present, appearing in March, July and September, but I should certainly have expected it in large numbers in every month of this period.

In October and November the entire stream is sterile. This occurs through the flushing given by the rush of water off the cart road and the high land on its northern side, which reaches this stream through culverts and contour drains. In December young *Anopheline* larvæ only were found, but all of them failed to reach maturity.

Paddy field H (Plate XXVI).—Like the majority of paddy fields in the hill-country of Ceylon, the water in this is never stationary, running continuously through the field, there being no means of shutting it off, even when the crop is ripening. The field is exposed, of course, to full sunlight. Larvæ were far from plentiful. None were found in March, when the *Maha* crop was ripening. In April, just before next ploughing, *C. tritæniorhyncus* was found in small numbers, both from the edges of the bunds closing the individual plots, and out in the middle of these. In June, with seedlings a month old just transplanted, a few *A. maculatus* and *Culiciomyia fusca* were taken close to the edge of the bunds. July and August, under the growing *Yala* crop, yielded no larvæ. In September, after reaping, a few *A. funestus v. listoni* and a single *C. fusca* were bred. Throughout the ensuing *Maha* growing period no larvæ were found until January, when the rice was three-quarters grown, when *A. maculatus* and *C. bitæniorhyncus* were found. In February, when the grain had set, *A. funestus v. listoni, A. sinensis, Culex tritæniorhyncus,*

STREAM G.



PADDY-FIELD H.—Stream G leads out of this near the coconut tree.

Site of S.-W. Monsoon pool F in river.

and the (in this month) almost ubiquitous *C. mimeticus* were found breeding.

This result is not in accordance with that arrived at by Gunasekera[2] in the Kurunegala district, which lies, but at a much lower elevation, in the low-country to the west of the range bounding the Matale valley. Though of the ten species of *Anophelines* found in paddy fields in Kurunegala no less than six are found in this paddy field and its outlet stream, of which four are proved carriers, their numbers are very small as compared with the results from other localities on the estate. The fact that carriers were taken in the following estates of the field—(*a*) seedlings just transplanted, (*b*) plants three-quarters grown, (*c*) grain set, (*d*) among stubble after reaping,—would seem to indicate that there is no reason why *Anophelines* should not be found throughout the year in double-cropped paddy fields, and their discovery here during four months of the year only is due either to insufficient searching (though, in that case, larvæ must have been very few thus to escape detection), or to the fact that during the major portion of the year more suitable breeding places are available. That among estates in the hill-country of Ceylon paddy fields are not the chief source of malaria I think Table I amply demonstrates.

There remains to be considered artificial collections of water breeding mosquitos. Such, on any estate when proper attention is paid to sanitation, should be few. It is almost hopeless to look for co-operation from the Tamil coolie in this or any other sanitary matter. He must be constantly supervised, the messes he (or rather she, for the women are the worst and most incorrigible offenders) makes must be cleaned up after him, and when things get too bad, punishments must be inflicted. On this estate four sweepers are employed to a population of little over 300 (including infants), giving an average of under 40 line-rooms per sweeper. This works out at about 800 sq. yards per man, as against the 8,000 sq. yards given by Das[3] as a sweeper's task. The sweepers on this estate, however, have in addition charge of two sets of latrines each.

In spite of the large scavenging staff employed, supervised by a daily inspection by the Estate Dispenser, a certain number of artificial collections of water have occurred during the period of the survey. These consists of old tins, broken chatties, etc., thrown into the vegetable gardens around the lines. During the course of the survey all those found to contain larvæ have been brought to the laboratory, and the contents bred out. The usual species have appeared. *Stegomyia*

scutellaris and its predator, *Armigeres obturbans*, regularly—whilst in the north-east monsoon *Stegomyia trilineata* and *Armigeres aureolineata* in addition are found. *Lophoceratomyia minutissima* bred in such collections also during April and May. In October, antiformicas in my bungalow that had not been recently kerosined yielded a heavy take of *S. scutellaris* only (the water still had a distinct smell and faint film of kerosine), whilst a garden water butt (also distinctly oily) proved to be breeding *S. sugens* only. This species is much less blood-thirsty than *scutellaris*, and the latter alone were in evidence in the bungalow. A blocked roof-gutter, in the bungalow in January, harboured *S. sugens* with a few *S. trilineata*. In November a large barrel (under shade), used by a coolie as a water butt for his vegetable garden at line 8, contained *Culiciomyia viridiventer* in large numbers, with a few *S. scutellaris*. This was the only locality, except the February droughtpools in Ravine C, in which *C. viridiventer* was found breeding. The absence of *Culex spp.* from such collections of water, when the results of the mosquito survey of Calcutta[20] are considered, is remarkable.

In a fully cultivated estate hollow trees containing more or less permanent collections of water are not to be expected, and the only instances that occurred were during the rainy months, November to January, when water collected in the fasciations of the trunk of a prone *Ficus religiosa* that has not yet decayed away. The fauna was very different to that of any other site. In November this yielded the beautiful *Orthopodomyia anopheloides*, *Cyathomyia brevipalpis*, *Ochlerotatus gubernatoris*, and a single *S. scutellaris*. It was then oiled and proved sterile in December, and though a strong smell of oil remained in January, it then yielded *C. brevipalpis* and many *Armigeres obturbans*, which would thus appear to be predatory on the former species.

Finally, in the February drought, the small stream provided by the overflow water from the factory engine cooling tanks yielded a single *A. maculatus*. The water at the point this larva was taken had lost its artificial temperature.

The ravine down which water used in manufacturing passes to the river otherwise proved sterile. A few yards below the point where this larva was taken it is joined by water used in washing rubber, and this is too highly polluted with the acetic acid used to coagulate the latex to support larvæ.

Table III shows the chances per cent of any pair of species being found breeding together.

Having now passed in review all the mosquito breeding places on the estate, a few notes on each species found may be of interest.

Anopheles culicifacies (Giles).—This species occurs in March only, in small numbers. It then occurs in full sunlight in the river-bed, and in deeply shaded springs among rocks and in wells. It appears to be rare in Ceylon. It was not recorded from the Island by James,[4] though its occurrence has been noted by Chalmers,[5] and by Gunasekera. Unlike the latter observer, I have not found it in paddy fields.

A. funestus v. listoni (Liston).—Occurs in February-March and August-September only, but in fair numbers. It breeds in the river, in ravines, in wells and in paddy fields.

A. jamesii. (Theo.).—A rare mosquito, occurring in March and April in small numbers only. It breeds in grass-edged streams in deep shade and in the river in full sunlight.

A. maculatus (Theo.).—The malaria-carrier, par excellence, of the Ceylon hill country. It breeds in every month of the year, and in every permanent breeding place except the river. I have also bred a single specimen, in November, from water in a coconut shell in a village near the estate. It would be interesting to know whether any other observer has recorded the Planarian predator found by me in wells.

A. minimus v. aconitus (Don.),—A very rare species—its occurrence here resting on a single specimen bred in February from stream C just below its outflow from the paddy field.

A. rossii v. vagus (Don.).—The local species is always *v. vagus*. Not common, in December, January, March-April and June only. It appears to prefer dirty water, coming from the swamp E and lower portion of stream G fand the closed pool in the river-bed in the north-east monsoon. The only clean-water locality in which it occurred was in the river-bed in April (when it was associated with *Culex bitæniorhyncus*, a dirty water species), and the well D in January.

As Edwards[6] has apparently not examined Grassi's type of *A. subpictus*, I do not consider the identity of the two spp. proved, and do not at present, therefore, change the name of this species.

A. sinensis (*Wied.*).—A rare species here, occurring in the larval state in February and April only, though I suspect it breeds in at least December as well (*vide infra*). It occurs in the river and in the paddy field. Its occurrence in the latter only when the grain had set, *i.e.*, when

the soil had not been disturbed for some months, confirms Swellengrebel and Graaf's [7] observation on the point.

I consider that Edwards[6] is quite unjustified in sinking this species under *A. hyrcanus* (Pallas). Brunetti[8] considers this name a possible synonym of *Culex mimeticus*. (In his paper there appears to be a misprint, 1871 for 1771). Pallas' type probably no longer exists—Edwards in any case has not examined it—and without it the point can never be proved. Wiedemann's name having stood for nearly a century I decline to change it.

A. karwari (James).—A very rare species, its occurrence here being confined to a single specimen in November, in the tiny upper rock spring B. Its occurrence there with *A. maculatus* differs from the experience of Swellengrebel and Graaf[7] in the Dutch Indies, who failed to find the two species breeding together.

Armigeres aureolineata (Leic.).—Occurs in November only, breeding in coconut shells. Leicester[9] bred it "from shells of a fruit in jungle."

Armigeres sp.—A single ♀ bred in the upper rock pool B in October. Major Christophers writes me that it is near *moultoni* (Edw.), but differs in several respects. It is probably new, and is, in any case, very rare.

A. obturbans (Wlk.).—Though this species was only bred in four months of the year the imago occurs in practically every month, entering houses to bite at sundown. It was bred from an old zinc bath, and from coconut shells, in which it was preying on *S. scutellaris* and *S. trilineata*, and from a tree hole, where its prey was *Cyathomyia brevipalpis*.

Stegomyia scutellaris (Wlk.).*—Common throughout the year, breeding mainly in artificial collections of water. It is always a nuisance in shady places under rubber at any time of day, and enters houses to bite at sundown, disappearing after dark.

Stegomyia sugens (Wied).—Not so common as the foregoing species. Outside Ceylon it does not appear to exist in the Oriental region, being an African species. As noted by Ingram[11] on the Gold coast, and Johnson[10] in Nigeria, it occurs in the largest numbers in pools among rocks. It is not nearly so blood-thirsty as *S. scutellaris*, and when both species were breeding about the bungalow in October it was *scutellaris* alone that was a nuisance. In spite of the enormous numbers issuing from the pool in the rocks in ravine A, it was only

* More correctly,—*S. albopicta*, Skuse.

A. scutellaris again that attacked by day in the field. I am not aware, however, whether, unlike the latter species, it is purely nocturnal.

Stegomyia trilineata (Leic.).—Not common, and only occurred in large numbers in a rock pool. It was bred in small numbers from a roof-gutter and a broken chatty. Not known to bite here, though Leicester[9] says it does so.

Ochlerotatus gubernatoris (Giles).—A rare species, bred only from a rock pool and a tree hole. As both of these localities were sterilised after a single examination each, I am unable to say whether it occurs in other than the two months indicated in Table II.

Lophoceratomyia mammilifer (Leic.).—A rare species, breeding by itself in the lower rock pool B in October only. It apparently requires somewhat peculiar conditions, as *A. maculatus*, which had been breeding previously in this situation, did not do so that month.

Lophoceratomyia minutissima (Theo.).—Common, but not taken in June, August and September. Breeds in rock springs, ravines and the river, as well as in artificial collections of water in coconut shells, etc. Does not breed in wells.

Lophoceratomyia uniformis (Theo.).—Very rare, a single specimen only taken, found breeding among rocks in ravine C with other species in February. It is recorded from Peredeniya[12] in May. Apparently confined to Ceylon.

Orthopodomyia anopheloides (Giles).—A tree-hole breeding species. Occurred only in November, but the locality was sterilized after examination. I have also bred it from black and filthy water in a stump of giant bamboo (*Dendrocalamus giganteus*) in a village near the estate, also in November. The larva is red—(like a small *Toxorhynchites*)—and rests motionless unless disturbed, when it flings itself through the water in similar manner to that genus. I do not know if it is predaceous, but suspect it to be so on *Psychodid* larvæ.

Cyathomyia brevipalpis (Giles).—Also a tree-hole breeding species. It occurred in November and January, being able to re-establish itself in the same locality within two months of its sterilisation with oil. It is apparently preyed on by *A. obturbans*.

Culex bitæniorhynchus (Giles).—The presence of the larvæ is usually a sign of foul water. It occurs in all months except May, October and December, being absent in the latter months probably through flushing of its breeding places by flood water. The well in ravine C, which would otherwise have carried it through these months, was closed

to it through sterilisation. It breeds in the above-mentioned dirty well, in swamp E, and in the lower (dirty) half of ravine C. It was found in cleaner localities in small numbers, the river in April and the paddy field (plants three-quarters grown) in January.

Culex tritæniorhyncus (Giles).—Not common. Found breeding in February to May only, in small numbers, though the imagines were a nuisance in the bungalow for a few days at the end of October, being probably blown in from outside the estate by the north-east monsoon, as no larvæ were found in that month. It breeds in the paddy field, in the clean portion of stream G. leading out of it, and in the river-bed.

Culex gelidus (Theo.).—A rare mosquito here. A single ♂, from the river in April, was the only specimen bred, though I have taken a ♀, probably wind blown, in the bungalow in October, and swept one by day from grass a few days later.

Culex halifaxii (Theo.).—Though only a single specimen was bred, from the dirty portion of ravine C in January, where it occurred in company with several other species, I have taken specimens, in the bungalow at night, and resting by day under leaves, in March, June, October, November, December and January. It is a rare species, none the less.

Culex mimeticus (Theo.).*—Occurs only in December to April, and in June. Appears to breed indifferently in both clean and dirty water, doing so in ravine C and its well, the swamp E, the dirty north-east monsoon pool in the river, in both portions of stream G, and in the paddy field in undisturbed conditions of water.

Culiciomyia fusca (Theo.).—Another species which prefers dirty water, breeding in the well in ravine C, the swamp E, both portions of ravine C, and the paddy field under disturbed soil conditions. It also took possession of spring well D after this had been cleared of *A. maculatus* by the Planarian in the north-east monsoon. Occurs sparcely in January-March, June and September only. It was among the other wind-blown species of *Culex* taken in the bungalow at the end of October.

Culiciomyia pallidothorax (Theo.).—Not common. Breeds in January, March, July and December in the lower rock spring B, the swamp E, and the dirty portion of stream G.

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* The species referred to as *mimeticus* is most probably the recently distinguished *mimulus* (Edw.).

Culiciomyia viridiventer (Giles).—Breeds in November and February only. Found in large numbers in a tub under shade in the former month, and sparsely in the latter in pools in ravine C. It appears to be a hill species, though its breeding places here are very different from those in which it was found by Giles[14] at Naini Tal. I cannot add any information on the subject of the ♀ biting, which Blanchard[15] states it does not do.

Uranotænia campestris (Leic.).—Not common, breeds in February, March, June and August in streams and rock springs. I have bred it from both the clean and dirty portions of ravine C. It does not breed in wells.

Table IV shows the different species occurring in each breeding place. In addition to the 29 above-noted species found breeding on the estate, a further seven species have been captured there, regarding which a few notes are desirable.

Anopheles barbirostris (*v. d. W.*).—A single ♀ was taken on a window in my bungalow at the end of February. This is one of the species noted by Gunasekera[2] as breeding in paddy fields in the Kurunegala district. It probably does so in this district also.

Toxorhynchites immisericors (Wlk.).—I have captured specimens of this species here in December, February, March and May. The February specimens (♀♀) were taken sucking flowers of *Mikania scandens* at dusk. I have bred it from larvæ in a bamboo stump from a village near the estate, the imago, a ♂, emerging after $5\frac{3}{4}$ days' pupal period early in December. The larvæ, only one of which reached maturity, were fed on larvæ of *Armigeres aureolineata*, *A. obturbans*, *Stegomyia scutellaris* and *Culiciomyia viridiventer*. They killed far more larvæ than they eat.

Toxorhynchites minimus (Theo.).—I captured two ♀♀ (the first known) of this very rare species in March and April 1919. I have not seen it again since. One was taken on *Lantana* scrub at dusk on the boundaries of the estate far from the portion dealt with in this paper, the other was taken flying in bright sunlight in my garden at 10 a.m. The species is apparently confined to Ceylon.

Ochlerotatus greenii (Theo.).—I have taken ♀♀ as follows:—In bungalow by day in January and March, and sucking flowers of *Mikania scandens* at dusk in February. The species is apparently confined to Ceylon, and is rare. The ♀ is not a blood-sucker.

Ochlerotatus albotæniatus (Leic.).—A single ♀ was taken on a window in my bungalow during the day time in December.

Culex fatigans (Wied.).—The absence of this species from the list of those breeding on the estate is one of the most striking facts elicited during the course of the survey. This is due, I am convinced, to the coolie latrines (pit system) not being waterlogged. In the tea districts around Adam's Peak, at elevations around 4,000 ft., where many of the latines are waterlogged, the species swarms, and I feel sure is the one responsible for the numerous complaints made by Planters' Associations in that area, on the compulsory erection of latrines on estates in Ceylon three years ago, that since this had been done, mosquitos had increased enormously, and curtains are now necessary in localities where before they had never been needed. It is noted as a cess-pool breeder in Manila.[16] That in some locality near this estate suitable breeding sites for this species are occasionally found is proved by the capture in the bungalow of a solitary ♀ in March.

Rachionotomyia aranoides (*Theo.*).—This species is a tree-hole breeder. It was bred in large numbers in November from bamboo stumps on native-owned land just across the estate boundary, and two ♂♂ were bred from water in a hollow *Erythrina* tree in a neighbouring village in the same month. I have seen imagines at flowers of *Mikania scandens* on the estate at dusk in February, and by day on windows in the bungalow. The ♀ does not bite.

Brunetti[17] places this genus in *Culicini*, the ♀ only of *R. ceylonensis*, the type species, being known. The latter species now proves to be only a synonym of *R. aranoides*, which was originally described as a *Wyeomyia*, which genus belongs in *Sabethini*, to which tribe Edwards[21] would also refer *Rachionotomyia*. As the latter has an unadorned metanotum, to do so means a redefinition of *Sabethini* involving its most striking character, and as this has apparently not been done, it seems preferable to refer the genus to *Aedini*, a view which is strengthened by *R. aranoides* having been (as *S. inornata*) placed in a genus *Squamomyia*, which Brunetti[17] allies to *Aedes* and Theobold[18] to *Uranotænia*, a position in accordence with its appearance.

This list of species reveals a very considerable Malayan element, amounting to 25 per cent. in the local *Culicid fauna*.

Anopheles minimus v. aconitus is known elsewhere from the Dutch East Indies, Malaya, Burma and Calcutta. *A. rossii v. vagus* is the

Malayan form, and is found in the Indian region only in Burma, Assam and Bengal. *Stegomyia trilineata* is known from Malaya and Kurseong, *Culex halifaxii* from Malaya and Queensland, whilst *Ochlerotatus albotæniatus*, *Lophoceratomyia mammilifer*, *Culiciomyia fusca*, *Uranotænia campestris* and *Rachionotomyia aranoides* are purely Malayan species not otherwise recorded from outside that region. This point, I think, is sufficiently interesting to be worthy of mention.

The most noticeable absentee from the list is *Stegomyia fasciata* (Fab.), which appears to exist in Ceylon only at Colombo and Trincomali.[19] I found it to be common in the rest-house at the latter place at dusk in October. Whatever danger, therefore, there may be of yellow fever getting a footing in Ceylon, it would, in any case, be confined to the seaboard. Why the species has been unable to extend inland, either into the densely populated districts behind Colombo, or into the jungle behind Trincomali, raises a most interesting problem no yet attempted of solution.

During the year of the survey the figures of malarial cases in each line were kept separately, and are shown in groups of adjacent lines, as indicated in fig. 1, in Table V, with, for the sake of comparison, the figures for a house in a nearby village where a kangany of this estate lives on land of his own with his family, in a house of his own construction, in and around which the sanitary conditions are beyond the control of the estate, and subject only to such inspection and control as the Government Sanitary staff may provide. The average monthly total over the six years ending March, 1920, are also given for purposes of comparison, though the figures are not strictly comparable, owing to line rebuilding, and the shifting of markedly unhealthy lines to better sites, that took place in 1916 and 1917. The population figures, which include every coolie, from infants upwards, are not known for years other than that of the survey, only coolies of working age being recorded. Average population figures are therefore not shown, but cannot vary greatly, as the estate labour force has throughout been kept at approximately a uniform strength. Malaria on the estate in the past has been of the most virulent nature, but in the last five years there have been no deaths attributed to it, and the majority of cases represent an incapacitation of the patient for only a few days at a time. For the year of the survey, in which the incidence is above the average, this involves only a daily average of three of the population absent from work owing to the disease.

In sampling breeding places, only a qualitative analysis was attempted, and the number of carriers bred in each month is calculated on the imagines (of both sexes), emerging from the breeding bowls. I have found that it is practically impossible to get young larvæ of *A. maculatus*, the principal carrier, to complete their metamorphoses in the laboratory, and the number bred per month is therefore further affected by the stage in which the insect was obtained. If the water sample consisted mainly of old larvæ and pupæ, far higher emergence figures would be obtained than if it consisted mainly of young larvæ, but none the less, if the total carriers of all species bred during a month is divided into the malaria figures of the month following, a rough concordance is obtained during six months of the year, which is sufficient, I think, to show that, if any very considerable departure of the value of this quotient from the normal is obtained, the operation of some other factor may be looked for. It will be seen from Table VI. where these figures are worked out, that in August, September and February the number of carriers bred is too large for the number of cases of malaria occurring, and whilst the first two of these months are the windiest of the year, which might cause the weak flying *Anophelines* to be swept away, this explanation fails for February at the commencement of the calm period between the monsoons. Again, the numbers of carriers bred in April, May and October are too low for the number of cases in May, June and November. The former are all fairly windy months, but no more so than June, July and November, in which the carrier figures accord well with the July, August and December cases, and the opposite supposition, that in April, May and October infected *Anophelines* are blown into the estates, would have to be advanced. If it could be shown that in these three months the low-lying lands around the estate in which the villages are situated were breeding *Anophelines* in large numbers, the first movements of the south-west monsoon in the first two of these months, and of the north-east monsoon in the third might result in a foreign-bred population of infected *Anophelines* arriving in the estate, whilst in the very windy August and September they may find the greatest difficulty in directing their flight at all, and so frequently perish, but this still leaves the quiet month of February unexplained. There is undoubtedly an uncomprehended factor at work. The foregoing theory is full of suppositions, but is advanced for what it is worth.

As regards figures for any one group of lines with reference to the emergences from the nearby breeding places, no special correlation can be obtained, and, owing to the fact that most of the distances are under

TABLE I

Breeding Results.

	1919. MARCH.	APRIL.	MAY.	JUNE.	JULY.	AUGUST.	SEPTEMBER.	OCTOBER.
Rain Inches Days.	5·42 6	5·95 13	8·98 18	4·02 22	3·95 21	3·26 23	8·88 19	7·89 21
Stream A.	Anopheles maculatus . . 2 Uranotænia campestris . . 1	*Nil.* (Examined on 2 days.)	(2 Anophelines and 1 Culicine bred. Damaged.)	Anopheles maculatus . 8	*Nil.*	Anopheles maculatus . 2	Uranotænia campestris 3 *Rainpool in rocks.* Stegomyia scutellaris . 126 „ sugens . 450 „ trilineata . 70 Ochlerotatus gubernatoris 3 (Filled in after sampling.)	Anopheles maculatus . Indeterminable Culicine .
Rock spring B.	Anopheles culicifacies . . 3 „ maculatus . . 12 Lophoceratomyia minutissima . 3 Culiciomyia palludothorax . . 3	Anopheles maculatus . . 3 (Oiled after sampling.)	Stegomyia sugens . . 1	Anopheles maculatus . 2 Uranotænia campestris 1	Anopheles maculatus . 2 Stegomyia scutellaris . 1 Culiciomyia palludothorax . 4	Anopheles maculatus . 3	Anopheles maculatus . 7	Armigeres sp. nov. (Cu. 31) Lophoceratomyia mammifer „ minutissima
Ravine C.	Anopheles maculatus . . 1 Culex mimeticus . . . 17	Anopheles maculatus . . 1 Culex mimeticus . . 3	Anopheles maculatus . . 5	Anopheles maculatus . 9 Culex bitæniorhynchus . 8	Anopheles maculatus . . 2 Culex bitæniorhynchus . 72	Anopheles maculatus . 15	Anopheles maculatus . 25	Anopheles maculatus .
Spring well D	Anopheles culicifacies . . 1 „ funestus v. listoni . 1 „ maculatus . . 4	*Nil.* [2 unhealthy larvæ (Anopheline) died.] (Cleaned out and wired over after examination.)	*Nil.*	Anopheles maculatus . 16	Anopheles maculatus . 21	Anopheles funestus v. listoni . 1 „ maculatus . 25	Anopheles funestus v. listoni . 1 „ maculatus . 9	Anopheles maculatus .
Swamp E.	Anopheles rossii v. vagus . 1 Culiciomyia fusca . . . 1	Anopheles maculatus . . 4 „ rossii v. vagus . . 7 (Oiled after sampling.)	*Nil.*	Anopheles maculatus . 4 „ rossii v. vagus 1 Culex bitæniorhynchus 3	Anopheles maculatus . . 2	Anopheles maculatus . 2 Culex bitæniorhynchus . 2 (Many larvæ of C. bitæniorhynchus died.)	Anopheles maculatus . 1 Indeterminable Culicine 1	*Nil.*
River-bed F.	Anopheles culicifacies . . 3	Anopheles jamesii . . . 1 „ rossii v. vagus . . 2 „ sinensis . . . 1 Culex bitæniorhynchus . . 1 „ tritæniorhynchus . . 1 „ gelidus . . . 1	In flood.	In flood.	In flood.	Anopheles funestus v. listoni . . 13	In flood.	In flood.
Stream G.	Anopheles funestus v. listoni . 6 „ jamesii . . . 2 „ maculatus . . 21 Lophoceratomyia minutissima . 1 Culex bitæniorhynchus . . 2 „ tritæniorhynchus . . 1 Culiciomyia fusca . . . 1	Anopheles maculatus . . 6	Anopheles maculatus . . 5 Culex tritæniorhynchus . 1	Culex mimeticus . . 3 Uranotænia campestris 1	Lophoceratomyia minutissima . 1 Culex bitæniorhynchus . . 1	Anopheles maculatus . 10	Anopheles maculatus . 12 Culex bitæniorhynchus 5	*Nil.* (Examined on 2 days.)
Paddy field H.	*Nil.*	Culex tritæniorhynchus . . 2	*Nil.*	Anopheles maculatus . 1 Culiciomyia fusca . 1	*Nil.*	*Nil.*	Anopheles funestus v. listoni . 3 Culiciomyia fusca . 1	*Nil.*
Rain water in hollow trees tins, chatties, etc., J.	*Old tin bath.* Armigeres obturbans . . 1 Stegomyia scutellaris . . 52	*Old bucket.* Stegomyia scutellaris . . 60 Lophoceratomyia minutissima . 5	*Coconut shells and tins.* Stegomyia scutellaris . . 83 Lophoceratomyia minutissima . 14	*Nil.*	*Nil.*	*Nil.*	*Nil.*	*Bungalow antiformicas* Stegomyia scutellaris . *Garden water-butt.* Stegomyia sugens . .

TABLE I

Breeding Results.

GUST.	SEPTEMBER.	OCTOBER.	NOVEMBER.	DECEMBER.	JANUARY.	1920. FEBRUARY.
26	8·88	7·89	14·67	9·02	4·49	0·70
23	19	21	23	19	11	3
aculatus . 2	Uranotænia campestris 3 *Rainpool in rocks.* Stegomyia scutellaris . 126 sugens . 450 ,, trilineata . 70 Ochlerotatus gubernatoris 3 (Filled in after sampling.)	Anopheles maculatus . . 3 Indeterminable Culicine . . 1	Anopheles maculatus . . 2	Anopheles maculatus . . 17 Stegomyia scutellaris . . 1	(1 Anopheline larva died)	Anopheles funestus v. listoni . 2 ,, maculatus . . 8 Lophoceratomyia minutissima 1
aculatus . 3	Anopheles maculatus . 7	Armigeres sp. nov. (Cu. 31) . 1 Lophoceratomyia mammifer . 3 ,, minutissima . 1	Anopheles maculatus . . 6 ,, karwari . . . 1	*Nil.*	Anopheles maculatus . 3	Anopheles maculatus . 6
aculatus . 15	Anopheles maculatus . 25	Anopheles maculatus . . 3	Anopheles maculatus . . 14 Lophoceratomyia minutissima . 1	(Larvæ present but all failed to emerge.)	Anopheles maculatus . . 4 Culex mimeticus . . 1 Culiciomyia fusca . . . 2	Anopheles maculatus . . 10 Lophoceratomyia minutissima . 1 ,, uniformis . 1 Culex bitæniorhynchus . . 3 ,, mimeticus . . 3 Culiciomyia viridiventer . . 3
funestus listoni . 1 culatus . 25	Anopheles funestus v. listoni . 1 ,, maculatus . 9	Anopheles maculatus . . 2	*Nil.*	*Nil.*	Anopheles rossii v. vagus . . 1 Culiciomyia fusca . . . 1	Culiciomyia fusca . . 2
aculatus . 2 orhynchus . 2 æ of C. ynchus died.)	Anopheles maculatus . 1 Indeterminable Culicine 1	*Nil.*	Anopheles maculatus . . 1	Culiciomyia pallidothorax . . 3	*Nil.*	Anopheles maculatus . . 7 Lophoceratomyia minutissima . 1 Culex mimeticus . . 1 Culiciomyia fusca . . 1
funestus v. . . 13	In flood.	In flood.	In flood.	Anopheles rossii v. vagus . . 1 Stegomyia scutellaris . . 1 Lophoceratomyia minutissima . 4 Culex mimeticus . . 2	*Nil.*	Lophoceratomyia minutissima . 17 Culex mimeticus . . 6
aculatus . 10	Anopheles maculatus . 12 Culex bitæniorhynchus 5	*Nil.* (Examined on 2 days.)	*Nil.*	(Small Anopheline larvæ present—but all died.)	Anopheles maculatus . . 1 ,, rossii v. vagus . . 4 Lophoceratomyia minutissima . 1 Culex hahfaxii . . . 1 Culiciomyia fusca . . . 1 ,, pallidothorax . 5	Anopheles maculatus . . 5 ,, minimus v. aconitus . 1 Lophoceratomyia minutissima . 12 Uranotænia campestris . . 7 Culex mimeticus . . . 13
il.	Anopheles funestus v. listoni . 3 Culiciomyia fusca . 1	*Nil.*	*Nil.*	*Nil.*	Anopheles maculatus . . 5 Culex bitæniorhynchus . . 2	Anopheles funestus v. listoni, . 2 ,, sinensis . . . 1 Culex bitæniorhynchus . . 2 ,, mimeticus . . 1
il.	*Nil.*	*Bungalow antiformicas* Stegomyia scutellaris . . 76 *Garden water-butt.* Stegomyia sugens . . . 34	*Coconut shells and old tin.* Armigeres obturbans . . 13 ,, aureolineata . . 53 Stegomyia scutellaris . . 32 *Garden water-butt.* Stegomyia scutellaris . . 7 Culiciomyia viridiventer . . 20 *Hollow tree.* Stegomyia scutellaris . . 1 Ochlerotatus gubernatoris . . 3 Orthopodomyia anopheloides . 8 Cyathomyia brevipalpis . . 1 (Oiled after sampling.)	*Broken chatty.* Armigeres obturbans . . 2 Stegomyia scutellaris . . 40 ,, trilineata . . 4	*Bungalow roof gutter.* Stegomyia sugens . . . 20 ,, trilineata . . . 2 *Hollow tree.* Armigeres obturbans . . 29 Cyathomyia brevipalpis . . 8	*Engine cooling tank overflow drain.* Anopheles maculatus . 1

TABLE II.

Monthly breeding totals for each species found.

Reference No. CU.	Identification.	March, 1919.	April.	May.	June.	July.	August.	September.	October.	November.	December.	January.	February, 1920.	Totals.	% of all Spp.	% of Anophelines.
14 C.	Anopheles culicifacies (G.)	7	..	..	..	..	..	..	..	..	..	..	..	7	·3	1·8
13 C.	,, funestus v. listoni (L.)	7	..	..	..	..	14	4	..	..	..	..	4	29	1·7	7·2
10	,, jamesii (Th.)	2	1	..	..	..	..	..	..	..	..	..	..	3	·2	·8
12 C.	,, maculatus (Th.)	40	14	10	40	27	58	54	8	23	17	13	36	340	18·6	85·0
43 C.	,, minimus v. aconitus (Don.)	..	..	..	..	..	..	..	..	..	..	..	1	1	·1	·2
11	,, rossii v. vagus (Don.)	1	9	..	1	..	..	..	..	..	1	5	..	17	1·0	4·3
20 C.	,, sinensis (Wd.)	..	1	..	..	..	..	..	..	..	..	..	1	2	·1	·5
36	,, karwari (J.)	..	..	..	..	..	..	..	..	1	..	..	..	1	·1	·2
35	Armigeres aureolineata (Lc.)	..	..	..	..	..	..	..	..	53	..	..	..	53	3·0	..
31	Armigeres sp. nov.	..	..	..	..	..	..	..	1	..	..	..	..	1	·1	..
1	,, obturbans (Wlk.)	1	..	..	..	..	..	..	..	13	2	29	..	45	2·6	..
2	Stegomyia scutellaris (Wlk.)	52	60	83	..	1	..	126	76	40	42	..	..	480	26·7	..
25	,, sugens (Wd.)	..	..	1	..	..	.	450	34	..	..	20	..	505	28·1	..
26	,, trilineata (Lc.)	..	..	..	..	..	..	70	..	..	4	2	..	76	4·2	..
27	Ochlerotatus gubernatoris (G.)	..	..	..	..	..	..	3	..	3	..	..	..	6	·3	..
32	Lophoceratomyia mammilifer (Lc.)	..	..	..	..	..	..	..	3	..	..	..	..	3	·2	..
4	,, minutissima (Th.)	4	5	14	..	1	..	..	1	1	4	1	32	63	3·6	..
44	,, uniformis (Th.)	..	..	..	..	..	..	..	..	..	..	..	1	1	·1	..
34	Orthopodomyia anopheloides (G.)	..	..	..	..	..	..	..	..	8	..	..	..	8	·4	..
39	Cyathomyia brevipalpis (G.)	..	..	..	..	..	..	..	..	1	..	8	..	9	·5	..
6	Culex bitæniorhyncus (G.)	2	1	..	11	3	2	5	..	..	..	2	3	29	1·7	..
21	,, tritæniorhyncus (G.)	1	3	1	..	..	.	..	..	..	..	..	2	7	·3	..
19	,, gelidus (Th.)	..	1	..	..	..	..	..	..	..	..	..	..	1	·1	..
7	,, halifaxii (Th.)	..	..	..	..	..	..	..	.	..	..	1	..	1	·1	..
8	,, mimeticus (Noe)	17	3	..	3	..	..	..	..	.	2	1	24	50	2·8	..
3	Culiciomyia fusca (Th.)	2	..	..	1	..	.	1	..	..	..	4	3	11	·5	..
9	,, pallidothorax (Th.)	3	..	..	..	4	..	..	..	..	..	5	..	12	·6	..
38	,, viridiventer (G.)	..	..	..	..	..	.	..	.	20	..	..	3	23	1·3	..
16	Uranotænia campestris (Lc.)	1	..	..	2	..	..	3	..	..	.	..	7	13	·7	.
	Total bred each month	140	98	109	58	36	74	716	123	163	72	91	117	1,797	100	100
	Total carriers bred each month	54	15	10	40	27	72	58	8	23	17	13	42	379	21·1	22·2
	No. of spp. breeding	14	10	5	6	5	3	9	6	10	7	12	12	..	Carrier % of total	Anopheline % of total.
	No. of spp. of carriers breeding	3	2	1	1	1	2	2	1	1	1	1	4	..		

C = Malaria carrier.

	Anopheles. culicifacies.
Anopheles culicifacies	33
,, funestus v. listoni	11
,, jamesii	..
,, maculatus	3
,, minimus v. aconitus	..
,, rossii v. vagus	..
,, sinensis	..
karwari	..
Armigeres aureolineata	..
Armigeres sp. nov.	..
,, obturbans	..
Stegomyia scutellaris	..
,, sugens	..
,, trilineata	..
Ochlerotatus gubernatoris	..
Lophoceratomyia mammilifer	..
,, minutissima	7
,, uniformis	..
Orthopodmyia anopheloides	..
Cyathomyia brevipalpis	..
Culex bitæniorhyncus	..
,, tritæniorhyncus	..
,, gelidus	..
,, halifaxii	..
,, mimeticus	..
Culiciomyia fusca	..
,, pallidothorax	25
,, viridiventer	..
Uranotænia campestris	..

TABLE III.

Chances % of any one sp. of Culicid being found breeding in the same location as any other sp.

	Anopheles culicifacies.	Anopheles funestus v. listoni.	Anopheles jamesii.	Anopheles maculatus.	Anopheles minimus v. aconitus.	Anopheles rossi v. vagus.	Anopheles sinensis.	Anopheles karwari.	Aëmigeres aureolineata.	Armigeres sp. nov.	Armigeres obturbans.	Stegomyia scutellaris.	Stegomyia sugens.	Stegomyia trilineata.	Ochlerotatus gubernatoris.	Lophoceratomyia mammilifer.	Lophoceratomyia minutissima.	Lophoceratomyia uniformis.	Orthopodmyia anopheloides.	Cyathomyia brevipalpis.	Culex bitæniorhynchus.	Culex tritæniorhynchus.	Culex gelidus.	Culex halifaxii.	Culex minutissimus.	Culiciomyia fusca.	Culiciomyia pallidothorax.	Culiciomyia viridiventer.	Uranotænia campestris.
s culicifacies .	33	33	..	66	..	..	..	..	..	..	..	..	..	..	..	..	33	..	..	..	..	..	..	..	..	33	..	..	..
funestus v. listoni .	11	11	..	67	..	..	11	..	..	..	..	..	..	..	..	..	11	..	..	..	..	22	..	..	11	22	..	..	..
jamesii . . .	..	..	..	50	..	50	50	..	..	..	..	..	..	..	..	..	..	..	..	..	50	50	50	..	..	..	..	..	..
maculatus . .	3	9	1·5	52	..	4·5	..	1·5	..	..	..	1·5	..	..	..	..	10·5	1·5	..	..	12	1·5	..	1·5	9	7·5	3	3	4·5
minimus v. aconitus .	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	100	..	..	.	..	..	..	..	100	..	..	..	100
rossi v. vagus .	..	..	14	43	..	..	14	..	..	..	..	14	..	..	..	..	29	..	.	..	29	14	14	14	..	43	14	..	..
sinensis . . .	..	50	50	..	..	50	..	..	..	..	..	..	..	..	..	..	..	..	..	..	50	100	100	..	100	..	..	..	..
karwari . . .	..	..	..	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
s aureolineata .	..	..	..	..	..	..	..	..	..	..	100	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
s sp. nov. . .	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	100	..	..	..	..	..	..	..	..	..	..	..	..
obturbans .	..	..	..	..	..	..	..	..	40	..	..	80	..	20	..	..	..	..	..	20	..	..	..	..	..	..	..	..	..
a scutellaris .	..	..	..	5·5	..	5·5	..	..	11	..	22	33	5·5	11	11	..	17	..	5·5	5·5	..	..	..	..	..	..	5·5	5·5	..
sugens . .	..	..	..	..	..	..	..	..	..	..	..	25	50	50	25	..	..	..	..	..	.	..	..	..	..	..	..	..	..
trilineata .	..	..	..	..	..	..	..	..	..	..	33	66	66	..	33	..	..	..	..	..	..	..	..	..	..	..	..	..	..
atus gubernatoris.	..	..	..	..	..	..	..	..	..	..	..	100	50	50	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
ratomyia mammilifer .	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
minutissima .	7	7	..	47	7	13	..	..	..	7	..	20	..	..	..	100	..	..	..	..	..	..	..	..	..	..	..	..	..
uniformis .	..	..	..	100	..	..	..	..	..	..	..	..	..	..	..	..	..	7	..	..	13	..	..	7	27	13	13	7	7
myia anopheloides .	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	100	..	..	..	..	..	..	..	..	..	..	100	..
yia brevipalpis .	..	..	..	..	..	..	..	..	..	..	..	100	..	..	100	..	..	..	..	100	..	..	..	..	..	..	..	..	..
æniorhynchus .	..	..	10	80	..	20	10	..	..	..	50	50	..	..	50	..	..	..	50	..	..	..	..	..	..	..	..	..	..
tæniorhynchus .	..	40	20	20	..	20	40	..	..	..	..	..	..	..	..	..	20	..	..	..	..	10	10	..	..	..	..	..	..
dus . . .	..	..	100	..	..	100	100	..	..	..	..	..	..	..	..	..	..	..	..	..	20	40	20	..	..	20	..	.	.
lifaxii . . .	..	..	..	100	..	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	100	100	..	..	100	..	..	..	.
metieus . . .	..	..	..	50	8	..	..	..	..	..	..	..	..	..	..	..	100	..	..	..	..	..	..	..	..	100	100	..	.
ia fusca . . .	..	22	..	44	..	22	..	..	..	..	..	..	..	..	..	..	33	..	..	..	..	..	..	..	8	17	..	.	17
pallidothorax .	25	..	..	75	..	50	..	..	..	..	..	25	..	..	..	..	22	..	.	..	..	11	.	11	22	11	11	..	.
viridiventer .	..	..	..	66	..	..	..	..	..	..	..	33	..	..	..	..	25	..	..	..	..	..	.	25	..	25	25	..	.
ia campestris .	..	..	..	50	17	..	..	.	..	..	..	..	..	..	..	..	33	33	..	.	..	..	.	..	33	..	..	..	.
																	17	..	.	..	..	..	.	..	33	..	..	..	17

TABLE IV.

Species breeding in each locality.

Stream A.	Rock springs B.	Ravine C.	Spring Well D.	Swamp E.	River-bed F.	Stream G.	Paddy field H.	Tins, Chatties, etc.	Hollow tree. Rock Pool.
Anopheles funestus *v.* listoni.	Anopheles culicifacies ..	Anopheles maculatus ..	Anopheles culicifacies ..	Anopheles maculatus ..	Anopheles culicifacies ..	Anopheles funestus *v.* listoni..	Anopheles funestus *v.* listoni.	Armigeres aureolineata ..	Armigeres obturbans.
„ maculatus ..	„ maculatus ..	Lophoceratomyia minutissima	„ funestus *v.* listoni	„ rossii *v.* vagus ..	„ funestus *v.* listoni..	„ jamesii ..	„ maculatus ..	„ obturbans ..	Stegomyia scutellaris.
Stegomyia scutellaris ..	„ karwari ..	„ uniformis	„ maculatus ..	Lophoceratomyia minutissima	„ jamesii ..	„ maculatus ..	„ sinensis ..	Stegomyia scutellaris ..	„ sugens.
Lophoceratomyia minutissima	Armigeres sp. nov. ..	Culex bitæniorhyncus ..	„ rossii *v.* vagus ..	Culex bitæniorhyncus ..	„ rossii *v.* vagus ..	„ minimus *v.* aconitus	Culex bitæniorhyncus ..	„ sugens ..	„ trilineata.
Uranotænia campestris ..	Stegomyia scutellaris ..	„ mimeticus ..	Culiciomyia fusca ..	„ mimeticus ..	„ sinensis ..	„ rossii *v.* vagus ..	„ tritæniorhyncus ..	„ trilineata ..	Ochlerotatus gubernatoris.
	„ sugens ..	Culiciomyia fusca ..	....	Culiciomyia fusca ..	Stegomyia scutellaris ..	Lophoceratomyia minutissima	„ mimeticus ..	Lophoceratomyia minutissima	Orthopodomyia anopheloides.
	Lophoceratomyia mammilifer	„ viridiventer ..	....	„ pallidothorax .	Lophoceratomyia minutissima	Culex bitæniorhyncus ..	Culiciomyia fusca ..	Culiciomyia viridiventer ..	Cyathomyi brevipalpis.
	„ minutissima ..				Culex bitæniorhyncus ..	„ tritæniorhyncus.			
	Culiciomyia pallidathoraox ..				„ tritæniorhyncus ..	„ halifaxii.			
	Uranotænia campestris ..				„ gelidus	„ mimeticus.			
					„ mimeticus ..	Culiciomyia fusca.			
						„ pallidothorax.			
						Uranotænia campestris.			
(5)	(10)	(7)	(5)	(7)	(11)	(13)	(7)	(7)	(7)

TABLE V.

Malarial cases in each line group.

Coolie lines No.	1919 APRIL.			MAY.			JUNE.			JULY.			AUGUST.			SEPTEMBER.			OCTOBER.			NOVEMBER.			DECEMBER.			JANUARY.			FEBRUARY.			1920. MARCH.		
	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%
1	28	17	60·7	25	13	52·0	32	13	40·6	31	10	32·3	30	8	26·3	28	4	14·3	28	5	17·9	27	7	29·6	27	5	18·5	27	4	14·8	23	3	13·0	23	3	13·0
2	96	45	46·9	92	62	67·4	89	25	28·1	89	35	28·1	89	23	25·8	89	10	11·2	89	14	15·7	89	31	34·8	92	23	25·0	92	20	21·7	83	12	14·4	83	10	12·0
3	42	19	45·2	42	30	71·4	41	16	39·0	41	12	29·3	35	14	40·0	35	3	8·6	35	6	17·1	41	13	31·7	41	6	14·6	40	7	17·5	32	5	15·7	38	3	5·2
4	31	1	3·2	27	19	70·4	30	16	53·3	28	12	42·9	28	5	17·9	28	4	14·3	28	2	7·1	22	12	54·5	22	7	31·8	22	6	27·2	28	3	10·7	28	3	10·7
5	19	15	79·0	16	10	62·5	18	6	66·7	17	3	17·6	17	8	47·1	17	3	17·6	17	3	17·6	16	8	50·0	16	6	37·5	16	2	12·5	18	2	11·1	18	1	5·5
6	81	31	38·3	67	51	76·1	78	36	46·2	77	25	32·5	76	11	14·5	76	7	9·2	77	10	13·0	69	21	30·4	69	14	20·3	69	20	29·0	61	17	27·8	72	9	12·5
7	27	18	66·7	23	18	78·2	25	11	44·0	24	7	28·3	24	4	16·7	24	3	12·5	26	9	34·6	23	9	29·1	23	5	21·7	23	3	13·0	22	2	9·1	22	2	9·1
8	6	5	83·3	6	4	66·7	6	2	33·3	6	1	16·7	6	1	16·7	6	2	33·3	6	0	0	6	2	33·3	6	1	16·7	6	0	0	6	1	16·7	6	0	0
TOTALS	330	151	45·8	298	207	69·5	319	125	39·2	313	105	33·5	305	74	24·2	303	36	11·9	306	49	16·0	293	103	35·2	296	69	23·3	295	62	21·0	273	45	16·5	310	31	10·0
Houses in village	10	7	70·0	9	6	66·7	10	6	60·0	10	3	30·0	10	4	40·0	10	5	50	10	5	50	9	5	55·6	9	3	33·3	9	3	33·3	9	1	11·1	9	2	22·2
6 years' average		89			108			91			96			71			52			52			69			50			62			44			60	

half a mile, none can be expected. Malarial figures can only be examined on the totals.

The results of Table VI are shown graphically in fig. 2.

During the period of the survey a record was kept of the *Anophelines* captured in my bungalow. None were taken in bedrooms, and no case of malaria occurred in the bungalow, neither myself nor those of my servants who live at the bungalow being attacked. Such captures as were made took place on windows of the verandah, above which runs an iron trelliswork for ventilation purposes when the windows have to be kept closed in windy months. Large numbers of Diptera, wind-blown, occur on the windows, and being a collector of Diptera in general, the window panes are frequently searched for specimens. During the two monsoons the windows facing those directions show, respectively, the largest Dipterous population, clearly indicating that species found thereon (except obviously house haunting-species), wind-blown through the trellis above, make for the windows as soon as they find themselves able, in a quiet atmosphere, to control their flight. I think, therefore, that the majority of the *Anophelines* thus captured entered the bungalow wind-blown, and not of their own volition seeking a meal. The fact that it is only in the north-east monsoon that *Anophelines* were taken is inimical to the theory advanced above that the winds at the commencement of the south-west monsoon carry them into the estate in large numbers.

TABLE VI.

Relation of number of malaria-carriers bred to cases of malaria occurring.

Month.	Carriers bred.	Cases of Malaria.	Cases ÷ Carriers of previous month.
March . .	54	..	..
April . .	15	151	2·8
May . .	10	207	13·8
June . .	40	125	12·5
July . .	27	105	2·6
August . .	72	74	2·7
September . .	58	36	0·5
October . .	8	49	0·8
November . .	23	103	12·9
December . .	17	69	3·0
January . .	13	62	3·6
February . .	42	45	3·5
March . .	..	31	0·7
	379	1,057	2·8

1919. November. 7 A.M. *A. maculatus.* ♀. Unfed.
November 28th. 7 A.M. *A. maculatus.* ♀. Fresh blood in stomach.
December 24th. 7 A.M. *A. sinensis.* ♀.
December 31st. 8 A.M. *A. maculatus.* ♀. In grip of hunting spider.
1920. January 6th. 7 A.M. *A. maculatus.* Unfed.
February 3rd. Noon. *A. funestus v. listoni.* ♂.
Februay 29th. 7 A.M. *A. barbirostris.* ♀.

Finally, I have to thank Major S. R. Christophers, C.I.E., I.M.S., of the Malaria Bureau, for the great help he has afforded me with regard to the identification of the various species, many of which it was quite impossible to name without reference to an authoritatively identified collection: even so, the identification of the three species of *Culiciomyia* is given with some reserve.

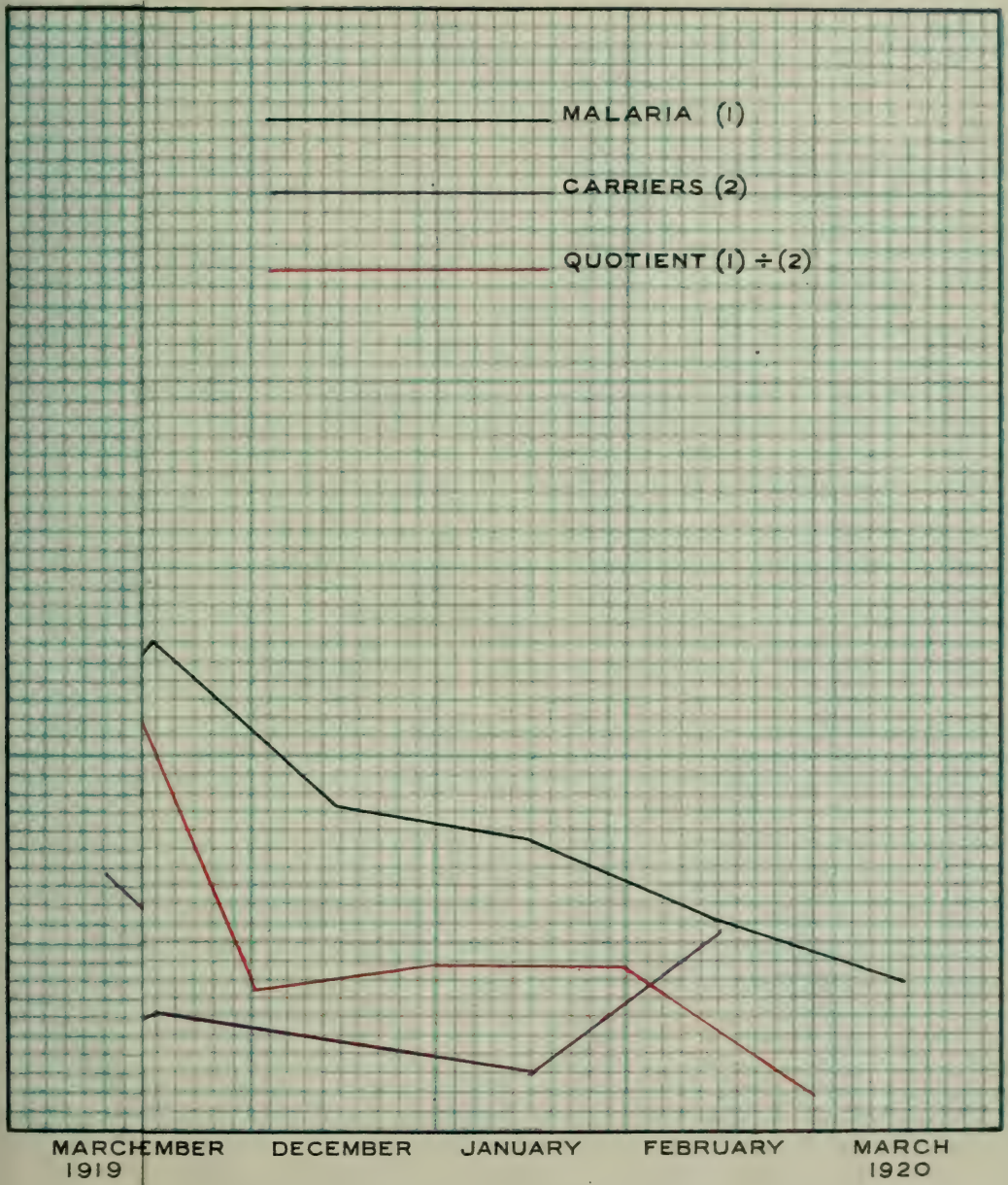
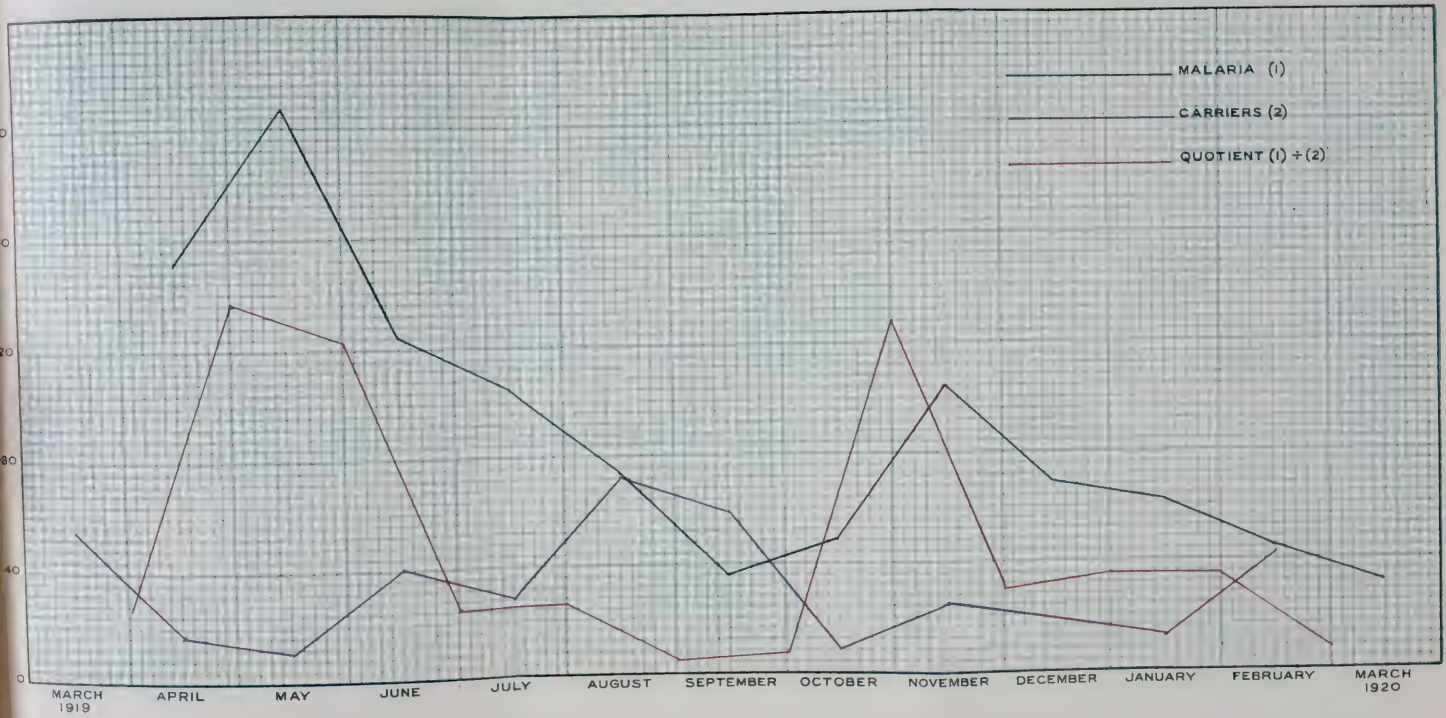

MALARIA (1)
CARRIERS (2)
QUOTIENT (1) ÷ (2)
MARCH 1919
DECEMBER
JANUARY
FEBRUARY
MARCH 1920

FIG. 2.


MALARIA (1)
CARRIERS (2)
QUOTIENT (1) ÷ (2)
0
40
80
120
160
200
MARCH 1919
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
JANUARY
FEBRUARY
MARCH 1920

Conclusions.

I. The Malayan element in the *Culicid* fauna of the lower hills of Ceylon is very marked.

II. The only malaria-carrier of importance on estates in this area is *A. maculatus*.

III. *A. maculatus* breeds almost entirely in natural collections of water, and the malarial incidence is insufficient to justify any very expensive measures for its eradication.

IV. The presence of *Culex fatigans* depends upon the presence of water in the latrines.

V. *Stegomyia fasciata*, the carrier of yellow fever, is entirely absent.

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OBSERVATIONS ON THE PATHOLOGY AND BACTERIOLOGY OF INFLUENZA.*

BY

LIEUT.-COLONEL E. D. W. GREIG, C.I.E., M.D., D.SC., I.M.S.,

Officiating Director, Pasteur Institute of India, Kasauli, Punjab.

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THE observations recorded in this paper were made at Karachi amongst the Indian troops during the autumn of 1918 and beginning of 1919. Since it was written I have studied the literature, recently published in America, dealing with the results of the pathological investigations in the various military camps in connexion with Influenza and Pneumonia. Further, I have had the advantage of seeing the specimens and discussing the results with various investigators during my recent visit to the United States of America. My conversations with Professor W. G. MacCallum at the Pathological Department, Johns Hopkins Hospital, were particularly instructive, as he himself has made a very extensive study of the pathology of Influenza and Pneumonia. In his important monograph on the Pathology of the Pneumonia in the United States Army Camps during the winter of 1917-18 (1) he clearly shows that the Pneumonia, associated with this great epidemic of Measles, had well-marked pathological features and was caused by a hæmolytic streptococcus. In a further paper (2) he distinguishes the pathology of Pneumonia following Influenza from that associated with Measles. I also had the benefit of discussing with Professor S. Burt Wolbach, of Harvard, this question and seeing his specimens. He investigated the cases at Camp Devens, Mass. (3)

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Whatever rôle we may attribute to such organisms as B. influenzæ, pneumococcus, hæmolytic streptococcus and others in the ætiology of respiratory disease, whether primary or secondary invaders, it should be remembered that each is associated with quite definite pathological features. Thus, from the lesions met with at the necropsy it is possible to differentiate a hæmolytic streptococcus from a B. influenzæ infection. Hence the importance of carefully conducted necropsies in cases of epidemic respiratory disease.

I made necropsies on 60 cases of fatal Influenza during the height of the second Influenza wave at Karachi in the autumn of 1918. The cases occurred amongst the Indian troops stationed there. As a result of a study of the pathological and bacteriological evidence obtained, it became evident that we were not dealing with a hæmolytic streptococcal infection, but with one associated with B. influenzæ and pneumoccus. In a previous paper (4) certain of the results of this investigation have been recorded, and in the present one the further pathological studies are dealt with.

Respiratory system.

As in all the cases this system was severely attacked, it is considered first.

(1) *Accessory Nasal Sinuses.*—Lesions met with in these places have been already fully considered in a previous paper (4). In this connexion it may be stated that a paper was published by E. Fraenkel(5) about the same time (January 1919) as ours, in which he states that he had not seen any account of sinusitis in Influenza. He examined 60 cases and found the sinuses pathologic in 75%. He does not know of any other acute infectious disease which involves the sinuses to the same extent. In 146 other cadavers, he found evidence of sinusitis in less than 50%. Later Stone and Swift, in their investigation of cases at Fort Riley, Kansas (6), have reported lesions of the sinuses. Wolbach (3) examined 20 cases and found infection of the middle ears in 13, infection of the sphenoidal sinus in 20, frontal sinus in seven and of the ethmoidal cells in eight cases. As in our observation, he found B. influenzæ and pneumococcus in the sinuses.

It is interesting to note that recently in Roumania (20) a tendency to sinus disease has been noted by Dr. Muntean, and he records that in a certain number of cases there results either a chronic sinusitis or a transmission of the infective process to the orbital or cranial cavities,

with occasional fatal termination. In December 1918 we (4) drew attention to these points and emphasized their practical importance.

(2) *Pharynx, Larynx, and Trachea.*—The results of the pathological observations are recorded in Tables Nos. 1—3.

TABLE No. 1

Pharynx.

	Number of cases.
MUCOUS MEMBRANE—	
1. Congested	30
2. Normal	20
TOTAL ..	50

TABLE No. 2.

Larynx.

MUCOUS MEMBRANE—	
1. Congested	45
2. Normal	9
TOTAL ..	54

TABLE No 3

Trachea

MUCOUS MEMBRANE—	
1 Congested	52
Normal	8
TOTAL ..	60

As will be seen, congestion of the upper respiratory passages was noted frequently. In a number of cases, the larynx and trachea were of a deep red colour, specially towards the lower part of the latter. A pseudomembrane was not observed in the larynx or trachea ; deep ulcerations of the vocal cords, in the pharyngeal and laryngeal walls, on epiglottis, on the aryteno-epiglottic folds, or in the pyriform sinuses, were never seen in my series of autopsies. MacCallum has recorded (1) such lesions in his necropsies on cases of streptococcal Pneumonia in Measles.

(3) *Bronchial Glands.*—The results of the observations are recorded in Table No. 4.

TABLE No. 4.

Bronchial Glands.

BRONCHIAL GLANDS—	
1. Enlarged	45
2. Not enlarged	5
3. Suppurating	1
TOTAL ..	51

Marked enlargement of these glands was noted frequently. The glands on section were very congested and engorged. In one case suppuration was observed.

In streptococcal Pneumonia following Measles, MacCallum (1) observes :—That the bronchial lymph glands are always somewhat enlarged, although not so greatly as in some other affections of the lungs. They are soft, œdematous, greyish-red except when deeply pigmented. He encountered no abscesses in these glands. Microscopically, the chief change is in the sinuses. These are very wide and packed with cells, chiefly large and small wandering cells, which have probably been swept into these sinuses from the lymphatics of the lungs, since they are of the type seen there. Among these are quantities of streptococci, often single or in pairs, often in tangled masses. As will be seen later, B. influenzæ was cultivated from the enlarged bronchial lymph glands. Streptococci were never cultivated.

(4). *Pleura.*—The results of the observations are recorded in Table No. 5.

TABLE No. 5.

Pleura.

	Number of cases.
PLEURA—	
1. Exudation without fluid	28
2. Exudation with clear fluid	3
3. Exudation with turbid fluid	1
4. Exudation with congestion	15
5. Exudation with petechial hæmorrhages ..	7
6. No exudation	3
TOTAL ..	57

It will be seen from the table that inflammation of the pleura was common. Adhesions were frequently met with. In many cases the surface appeared markedly congested and in some petechial hæmorrhages were noted. Effusion of fluid was rarely recorded and true empyema was not seen. There was thus no intense infection of the pleura. This latter observation is important, because in streptococcal bronchopneumonia it is of frequent occurrence. Thus MacCallum, in his monograph (1), states :—'The tabulation and analysis of 53 cases of all types of pneumonia as they occurred show an extraordinary frequency of pleurisy with fibrinopurulent effusion which is especially due to the activity of the hæmolytic streptococcus.' Also, 'At the autopsies fluid was found in quantities varying from 10 to 1,000 c.c.,

but it is possible that this does not represent the extremes. 1,000 to 1,500 cc. were commonly found.'

It will be noted, therefore, that, as regards the pleura, the pathological appearances found in influenzal Pneumonia differ strikingly from those of streptococcal bronchopneumonia. MacCallum (2) further points out that, in reference to Pneumonia produced by Bacillus influenzæ of Pfeiffer :—'The lymphatic channels in the bronchial walls and widened interlobular septums are inconspicuous, and none are found distended with exudate. There is no intense infection of the pleura or great outpouring of exudate. These two facts, together with the relatively inconspicuous part played by the polymorphonuclear leucocytes in the alveolar exudate, and in the exudate infiltrating the walls of the bronchi and alveoli, are all that distinguish this pulmonary change from the interstitial bronchopneumonia caused by the hæmolytic streptococcus as described in previous papers.' The sharp contrast between this form and those produced by the pneumococcus and streptococcus is very evident. Other American observers have described empyema associated with Influenza ; thus, Ballin (7) at Camp Grant, III, found that empyema develops in 4 weeks from date of onset. Stone and Swift (6) at Fort Riley, Kansas, in 55 autopsies found pleurisy with effusion 15 times, with suppuration four times. Lecount (8) found from 600 to 930 grammes by weight of fluid in the pleural cavities ; it was quite clear. post-influenzal empyema did occur. Goodpasture (9) in 16 autopsies on bronchopneumonia, due to hæmolytic streptococci following Influenza, found that in 11 cases empyema was present and four had prominent lymphatics. At the Walter Reed General Hospital, Lyon (10) made 56 necropsies on Influenza cases and found empyema only in two instances. Lamb (11) found empyema in post-influenzal Pneumonia in 65 out of 624 Pneumonias, twice as common as in post lobar Pneumonia. Blanton, Burham and Hunter (12), in their studies at Camp Custer, Mich., found that, coincident with the bronchopneumonia of the winter of 1918, very acute and fatal suppurative pleurisies were encountered. Strikingly different were the pleural infections encountered in the wake of the Influenza epidemic. The proportion of all Pneumonias developing fluid in the chest was nine times as great during January, February, March and April as October 1918. Wolbach (3) notes that 'The involvement of the pleura in lungs infected solely with the Influenza bacillus is very slight.' Empyema was found in cases secondarily infected with streptococcus or pneumococcus.

5. *Lungs.*—The results of the observations on the lungs are recorded in Table No. 6.

TABLE No. 6.

Lungs.

		Patches of lobular consolidation occurs in both cases.
1.	Acute bronchiolitis and peribronchiolitis with clear œdematous fluid	38
2.	Acute bronchiolitis and peribronchiolitis with purulent fluid	13
3.	Lobar consolidation	7
4.	Infarction-like appearance	1
	TOTAL ..	59

Two cases of (1) showed bronchiectasis and cavity formation.

Four cases of (2) showed cavity formation (one tubercular).

On section the lungs presented a very characteristic appearance, the mucous membrane of the small bronchioles was intensely congested, being dark-red in colour ; in some cases the fluid which exuded from the cut surface was abundant, clear and frothy, in others it had a creamy purulent appearance. Round the bronchioles there was a well-marked area of inflammation ; sometimes these areas stood up separate from one another, in other cases they had coalesced. A great deal of fluid escaped from the remainder of the cut surface of the lungs. Lobar Pneumonia was not noted frequently, but in a number of cases small patches of lobular consolidation were seen. In six cases definite cavity formation (bronchiectasis) was present ; in one case this was due to the tubercle bacillus which was found to be present in large numbers on microscopical examination. In one case an infarction-like appearance was observed.

Wolbach (3), from his study of the pathology of fatal cases of Influenza at Camp Devens, Mass., considers that :—' The condition, in fact, is one of panbronchitis ; peribronchitis with extensive infiltration of the interlobular septa ; and organization in alveoli and bronchioles.' Further he states : ' Without going into corroborative details at this time, I may say that in the gross appearance of the lungs I have laid emphasis upon, B. influenzæ was the only organism which could be cultivated and I unhesitatingly associate these distinctive conditions with this organism.' He lays stress on a condition of acute alveolar emphysema and states :—' It is this acute alveolar emphysema, with serous and hæmorrhagic exudate, that gives the characteristic gross appearance to the lungs in the

early stage of the disease.' 'It is the one distinctive feature in the pathology of Influenza Pneumonias, and its constant occurrence is indicative of the entity of the initial lung infection.' He considers the major injury is to the bronchial system and mainly in the finest bronchioles and alveolar ducts. 'Thus the patient is virtually blowing bubbles in his own lungs, into a medium of exudation relatively poor in fibrin.'

I have noted the injury to the bronchial system as evidenced by bronchiectasis, but I have not observed in my cases the extensive emphysema described.

Lecount (8), in autopsies on his cases of bronchopneumonia, found a relatively small amount of pneumonic consolidation, and that the periphery of the lung was chiefly affected in influenzal Pneumonia. Goodpasture (9) considers that bronchopneumonia following Influenza has at present absolutely no etiologic or pathologic definition; the type of organism and lesion is variable. Lyon (10), from a study of his autopsies at the Walter Reed Hospital, describes the lung lesions as hæmorrhagic pneumonitis. Lamb and Brannin (13) at Camp Cody, N.M., found peribronchial consolidation in 16 autopsies.

(6). *Heart.*—The results of the observations are recorded in Table No. 7.

TABLE No. 7.

Heart.

	Number of cases.	
Pericarditis	0	
Endocarditis	0	
Dilatation	7	(2 atheroma of first part of aorta.)
Muscle Wall flabby	28	(4 do. do.)
Heart healthy	20	
	—	*Petechial hæmorrhagic spots* in three cases on the visceral pericardium. *Discoloured clot* noted in the cavities of heart in 38 cases.
TOTAL ..	55	
	—	

As shown in the table, pericarditis was not observed in any of the cases. A flabby condition of the muscular wall, specially of the right ventricle, was the most noticeable deviation from normal in the heart. In a few cases petechial hæmorrhages were seen on the visceral pericardium. Endocarditis was not observed in any of the cases. White clot was frequently seen in the cavities of the heart and it extended

in some cases into the aorta and pulmonary artery. MacCallum (1), in his cases of streptococcal Pneumonia, observed a definite pericarditis in 10 out of 60. Endocarditis was noted in one case and the pneumococcus was isolated from the vegetations. Wolbach (3) states that :—'The heart muscle rarely has shown any gross or microscopic lesion.' Stone and Swift (6), in 55 autopsies, found dilatation of the heart in 38 and pericarditis in five. Lyon (10), at the Walter Reed Hospital, in 56 autopsies did not observe an instance of pericarditis; petechial hæmorrhagic spots were noted eight times on visceral and four times on parietal pericardium. In 27 cases the heart was moderately or slightly dilated, otherwise no change. White thrombi were frequently seen. Lamb and Brannin (13), at Camp Cody, noted no gross change in the heart and pericardium. Blanton, Burham and Hunter (12), in their studies on streptococcic infections at Camp Custer, encountered infections of the pericardium in a small proportion of cases.

We see that, as in the case of the pleura, the pericardium is more frequently infected in cases due to the hæmolytic streptococcus than in those associated with B. influenza infection, and thus we have another gross pathological distinction between the two conditions.

Peritoneum.

In none of the cases observed by me was peritonitis found to be present nor was the peritoneal fluid increased in amount. MacCallum (1), in his autopsies on cases of streptococcal Pneumonia, found :—'The peritoneal cavity was infected, however, in several cases, so that acute peritonitis seemed to be a not infrequent accompaniment of the streptococcal Pneumonia.' Here again is a distinction between the streptococcal and influenzal type of infection. Stone and Swift (6) observed peritonitis twice in 55 autopsies.

Blanton, Burham and Hunter (12) recorded that peritonitis was a comparatively unusual finding.

Alimentary Tract.

The results of the observations are recorded in the following Tables —Nos. 8 to 18.

Table No. 8.

Oesophagus.

	Number of cases.
Mucous Membrane—	
Congested	8
Not congested	39
Total ..	47

TABLE No. 9.

Stomach.

	Number of cas
MUCOUS MEMBRANE—	
Congested.	
(*a*) Without hæmorrhagic petechial spots ..	36
(*b*) With	18
Not congested	4
TOTAL ..	58

TABLE No. 10.

Duodenum.

	Number of cases.
MUCOUS MEMBRANE—	
Congested.	
(*a*) Without hæmorrhagic petechial spots ..	46
(*b*) With	3
Not congested	9
TOTAL ..	58

TABLE No. 11.

Jejunum.

	Number of cases
MUCOUS MEMBRANE—	
Congested	29
Not congested	29
TOTAL ..	58

TABLE No. 12

Ileum.

	Number of cases
MUCOUS MEMBRANE—	
Congested.	
(*a*) Without hæmorrhagic petechial spots ..	38
(*b*) With	1
Not congested	19
TOTAL ..	58

TABLE No. 13.

Cæcum.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	42
Not congested	16
TOTAL ..	58

TABLE No. 14.

Ascending Colon.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	42
Not congested	16
TOTAL ..	58

TABLE No. 15.

Transverse Colon.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	25
Not congested	33
TOTAL ..	58

TABLE No. 16.

Descending Colon.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	33
Not congested	25
TOTAL ..	58

TABLE No. 17.

Sigmoid.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	30
Not congested	28
TOTAL ..	58

TABLE No. 18.

Rectum.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	28
Not congested	30
TOTAL ..	58

From the stomach downward it will be noted that congestion with and without the presence of petechial spots was observed. These changes cannot be regarded, I think, as *post mortem*, but must have

occurred during life. The results of animal experiments, which will be detailed, support this view. The investigations of Lyon [10], at the Walter Reed Hospital, showed that similar lesions were found by him in his autopsies : although inclined to attribute them to *post-mortem* changes, he considers that there must have been some exudation during life. In two of his cases there was multiple ulceration of the stomach. The solitary follicles of the ileum were more or less enlarged.

In certain cases Influenza assumes clinically an 'abdominal type' and the lesions found at the autopsies show that a pathological change had occurred in the alimentary tract. Rosenow [14], in his studies in Influenza and Pneumonia, describes two cases of 'gastro-intestinal' Influenza and the reproduction of this condition experimentally in animals.

(7) *Liver.*—The following Table No. 19 records the results of the observations :—

TABLE No. 19.

Liver.

	Number of cases.	
Enlarged	9	(3 advanced fatty) (4 early fatty) (1 fibrous) (1 hydatid cyst)
Not enlarged	48	(29 early fatty change)
TOTAL ..	57	

With the exception of some fatty change there was no definite lesion of the liver.

MacCallum [1] also finds that the liver in most instances showed a moderate cloudy swelling, but otherwise no departure from normal. Lecount [8] notes that there is superficial fatty change. Lyon [10] records that the liver is pale, light yellowish-brown in colour.

(8) *Pancreas.*—The following Table No. 20 records the results of the observations :—

TABLE No. 20

Pancreas.

	Number of cases
ON SECTION—	
Congestion	21
No congestion	33
TOTAL ..	54

On section of the pancreas, congestion was noted, but otherwise no change was observed. Lyon ([10]) records similar results.

(9). *Spleen.*—The results of the observations are recorded in Table No. 21.

TABLE 21.

Spleen.

	Number of cases.
Enlarged	.. 21 (2 perisplenitis.)
Not enlarged	.. 36 (5 „)
TOTAL	.. 57

Considering that the population examined had all been exposed to the possibility of malarial infection, the changes in the spleen were slight.

Wolbach ([3]) also finds that 'the reaction of the spleen is very slight.'

MacCallum ([1]), in his streptococcal cases, notes :—'The spleen in the cases of streptococcal Pneumonia was sometimes slightly enlarged and occasionally rather soft. On the whole, however, it showed no definite change from normal, and microscopic examination failed to reveal any marked alteration of any part of its structure.' Lecount ([8]) notes that the spleen is not so enlarged as in lobar Pneumonia. Lyon ([10]) records enlargement in 12 out of 56 autopsies.

(10). *Kidneys.*—The following Table No. 22 records the results of the observations :—

TABLE NO. 22.

Kidneys.

	Number of cases.
Congestion	.. 20 (1 cystic.)
Cloudy swelling	.. 3
Contraction	.. 9 (3 cystic.)
Healthy	.. 19
TOTAL	.. 51

On section of the kidneys, congestion was the most frequent change observed. In a few cases cloudy swelling was noted. The congestion was, as a rule, well marked and the blood dripped freely from the cut surface.

MacCallum ([1]), in his cases of streptococcal Pneumonia, records that :—'In general, the kidneys were normal in appearance, except for slight cloudiness of the cortex which appeared in microscopic section as a slight swelling of the epithelial cells of the convoluted tubules.' He had

four cases of acute nephritis, but two of these were essentially pneumococcus infections. Stone and Swift ([6]), at Fort Filey, record 23 cases of parenchymatous nephritis out of 55 autopsies. Lecount ([8]) records cloudy swelling of the kidneys regularly. Lyon ([10]) observed well-marked congestion in nearly all the cases.

(11) *Suprarenals.*—The following Table No. 23 shows the results of the observations :—

TABLE No. 23.

Suprarenal Capsules.

	Number of cases.
ON SECTION—	
Congestion	2
Cystic	5
Normal	7
TOTAL ..	14

Section of the capsules showed no very definite pathological lesion.

Wolbach ([3]) states that lesions of the adrenal, when extensive, such as hæmorrhage, can be attributed to secondary infection, usually hemolytic streptococcus. Minor acute lesions are constantly found in the cortex in Influenza cases, but these lesions are similar to those found in many infectious diseases—the disappearance of lipoid content, and focal necrosis with mononuclear phagocytic cell reaction. Lyon ([10]) found no microscopic change in the suprarenals.

(12) *Urinary Bladder.*—The following Table No. 24 shows the results of the observations :—

TABLE No. 24.

Urinary Bladder.

	Number of cases.
MUCOUS MEMBRANE—	
Congested	25
Not congested	26
TOTAL ..	51

In about half the cases examined, congestion of the mucous membrane was noted, otherwise no change. Lyon ([10]) found no pathological change in the urinary bladder.

(13) *Testes.*—They were not systematically examined by me, but changes have been noted by other observers. MacCallum ([1]) states :—'The testes show in nearly all these cases an extraordinary change which especially striking in streptococcal infections, although occurring

sometimes in pneumococcal infection and failing to appear in some of the streptococcal ones. It appears to consist in a cessation of spermatogenesis, which has probably come about through a stoppage of mitosis at an early stage. In most of the cases of streptococcal infection spermatozoa are not found in the tubules of the testes, although a few may persist in the seminal vesicles embedded in the viscous fluid which is found there.' 'Cessation of spermatogenesis is so constant that it must be ascribed to the present disease.' Wolbach ([3]) records that :— 'The testes occasionally showed minute petechiæ, but on the whole no striking gross change was observed. Microscopically very striking changes were encountered in nearly every case, namely, the cessation of activity in the semeniferous tubules ; actual degenerative changes were frequently noted, and in late cases beginning fibrous tissue replacement of the degenerated tubules. This lesion of the testes seems to be wholly a toxic one, as there is very little cellular reaction.' 'The pathological changes found in the testes in epidemic pneumonia have been fully studied by Mills ([15]). Lyon ([10]) observed no gross change in the testes.

(14) *Thyroid.*—The following Table No. 25 shows the results of the observations :—

TABLE No. 25.

Thyroid.

	Number of cases.
Definitely enlarged	2 (1 suppurating.)
Not definitely enlarged	56
TOTAL ..	58

In one case suppuration of both lobes of the thyroid was noted, in another it was definitely enlarged, in all the other cases there was no abnormal naked-eye change.

(15) *Thymus.*—Lyon ([10]) noted enlargement in three of his cases.

(16) *Rectus Muscles.*—The following Table No. 26 shows the results of the observations :—

TABLE No. 26.

Rectus Muscles.

	Number of cases.
Hæmorrhages into both—	
With degeneration of fibres	2
Normal	56
TOTAL ..	58

The condition of the rectus muscles was interesting; in two cases there was hæmorrhage with degeneration of the fibres, in the other cases, to the naked eye, the muscle appeared normal.

Wolbach (3) notes that at Camp Devens :—'A number of these cases showed rupture and extensive hæmorrhage into the rectus muscle. This lesion has been noted in other muscles; for instance, the transversalis, the internal and external oblique, the latissimus dorsi, pectoralis major and the intercostal muscles.' He considers the lesion is produced by toxic action and finds it difficult to understand why such severe toxic action of the muscle and testes should occur, in the absence of effects, attributable to toxins, in other organs. MacCallum (1) records cases of this lesion of the rectus muscle. The fibres of the muscle show an extensive hyaline degeneration.

(17) *Brain and Meninges.*—The following Table No. 27 records the results of the observations :—

TABLE No. 27.

Brain and Meninges.

Normal	..11		
Congested and Œdematous ..	..37	(C.S.F. pressure plus.)	Clear Fluid 4 Turbid Fluid 9
TOTAL	48		

In a previous paper (4) the bacteriology of the cerebro-spinal fluid in influenza cases was dealt with by me.

It will be noted that 13 cases showed a distinct increase of cerebro-spinal fluid, in four of these the fluid was clear and in nine it was turbid. In 37 cases, the brain was œdematous and congested. The brain itself, naked eye, did not show any marked pathological change.

Stone and Swift (6) in 55 cases found meningitis in 24 and œdema in nine.

Lecount (8) found œdema but not so marked as in uræmia or heat-stroke.

Lyon (10) found œdema in seven cases and in one clouding of the pia-arachnoid.

Bacteriology.—Cultures were made from the organs at the time of the autopsy. The medium used for cultivating the organism was nutrose blood (human) agar. At the same time slides were prepared and examined after staining. The opportunities for bacteriological investigation were favourable, as the autopsy in most cases was made a few hours after death.

TABLE No. 28.

following Table shows the duration of the illness, the character of the cells in the contents of the Bronchioles, the presence or absence of B. influenzæ and pneumococcus in the contents of the Bronchioles and Bronchial Glands in cases of Influenza.

se. o.	Duration of illness.	BRONCHIOLES.					BRONCHIAL GLANDS.			
		MICROSCOPICAL.			CULTURAL.		MICROSCOPICAL.		CULTURAL	
		Cells.	B. Influe.	Pneu.	B. Influe.	Pneu.	B. Influe.	Pneu.	B. Influe.	Pneu.
1	....		0	0	0	0	0	0	0	0
2	....		+	+	+	+	0	0	0	0
5	....		+	−	+	−	0	0	+	0
6	....		0	0	+	+	0	0	0	0
7	....		0	0	0	0	0	0	+	−
8	....		0	0	+	−	0	0	+	−
9	....		0	0	0	0	0	0	+	−
10	9 days		0	0	−	−	0	0	0	0
11	13 ,,		0	0	−	−	0	0	−	−
12	20 ,,		0	0	0	0	0	0	0	0
13	16 ,,		+	+	+	−	0	0	−	+
14	13 ,,		−	+	0	0	0	0	−	−
15	20 ,,	P.N. +++	+	+	−	−	−	−	−	−
16	16 ,,	0	0	0	−	−	0	0	−	−
17	8 ,,	P.N. +++	+	+	−	−	−	−	−	−
18	27 ,,	P.N. ++	+++	+	+	−	−	−	−	−
19	9 ,,	P.N. ++	+	+	−	+	−	−	−	+
20	....	M.N.+ P.N. +++	+	−	−	+	−	−	−	−
21	11 days	P.N. +++	+	+	+	−	−	−	+	−
22	9 ,,	C.C.+ P.N. +++	+	+	+	−	+	−	+	−
23	17 ,,	0	0	0	0	0	0	0	0	0
24	13 ,,	0	0	0	0	0	0	0	0	0
25	13 ,,	C.C.	+	+	+	+	−	−	−	−
26	....	0	0	0	−	−	0	0	−	−
27	6 days	P.N. +++	+	+	−	−	0	0	−	−
28	8 ,,	P.N. +++	+	+	−	−	−	−	−	−
29	....	0	0	0	−	−	0	0	0	0

Symbols.

+ = Present. P.N. = Polynuclear cells.
− = Absent. M.N. = Mononuclear cells.
0 = Not examined. +++ = Cells numerous.
C.C. = Columnar cells. ++ = Cells less numerous.
+ = Cells scanty.

TABLE No. 28.—*Contd.*

Case No.	Duration of illness.	BRONCHIOLES.					BRONCHIAL GLANDS.			
		MICROSCOPICAL.			CULTURAL.		MICROSCOPICAL.		CULTURAL.	
		Cells.	B. Influe.	Pneu.	B. Influe.	Pneu.	B. Influe.	Pneu.	B. Influe.	Pneu.
31	4 days	P.N. ++	−	−	0	0	0	0	0	0
33	5 ,,	0	−	−	−	−	−	−	−	−
34	....	0	−	+	−	−	0	0	−	−
36	....	0	+	−	++	−	−	−	+	−
37	15 days	0	0	0	0	0	0	0	0	0
38	13 ,,	0	0	0	0	0	0	0	0	0
39	19 ,,	P.N. +++	+	+		−	−	−	−	−
41	9 ,,	0	+	−	−	−	−	+	−	−
42	....	P.N. +	+	−	+	−	−	−	+	−
43	7 days	P.N. ++	−	+	−	+	−	−	−	−
44	7 ,,	P.N. ++	+	+	+	−	−	−	0	0
46	10 ,,	0	−	−	+	−	−	−	0	0
47	8 ,,	P.N. +++	+	+	+	+	−	+	−	−
48	7 ,,	M.N. + P.N.++	+	+	+	+	−	−	+	+
49	10 ,,	P.N. ++	+	+	+	+	−	−	−	−
51	11 ,,	M.N. + P.N.+	+	+	+	+	−	−	0	0
52	12 ,,	0	+++	+	+	−	−	−	+	−
53	10 ,,	P.N. +	+	+	−	−	−	+	−	−
54	10 ,,	P.N. +	−	+	−	+	−	+	−	+
55	10 ,,	P.N. +	+	+	+	−	−	−	−	−
56	11 ,,	P.N. +	+	+	−	+	−	−	0	0
57	14 ,,	P.N. +	+	+	−	+	−	−	−	−
58	15 ,,	P.N. +	+	+	−	+	+	−	−	−
59	11 ,,	P.N. +	+	+	−	+	+	−	−	−
60	12 ,,	P.N. +	−	+	−	+	−	−	−	−
61	13 ,,	P.N. +++	−	+	−	+	−	0	0	0
62	16 ,,	P.N. +++	−	+	−	+	0	0	0	0

Symbols.

+ = Present.
− = Absent.
0 = Not examined.
P.N. = Polynuclear cells.
M.N. = Mononuclear cells.
+++ = Cells numerous.
++ = Cells less numerous.
+ = Cells scanty.

TABLE No. 28.—*Concld.*

Case No.	Duration of illness.	BRONCHIOLES.					BRONCHIAL GLANDS.			
		MICROSCOPICAL.			CULTURAL.		MICROSCOPICAL.		CULTURAL.	
		Cells.	B. Influe.	Pneu.	B. Influe.	Pneu.	B. Influe.	Pneu.	B. Influe.	Pneu.
63	....	P.N. +	−	+	−	+	−	−	−	+
64	13 days	P.N. +	+	+	−	+	0	0	−	+
65	5 ,,	P.N. +	−	+	+	+	−	−	+	+
66	11 ,,	P.N. +	−	−	−	+	0	0	−	+
67	10 ,,	P.N. ++	+	+	+	+	+	+	+	+
68	18 ,,	P.N. ++	+	+	+	+	+	−	+	+

Symbols.

+ = Present.
− = Absent.
0 = Not examined.
P.N. = Polynuclear cells.
++ = Cells less numerous.
\+ = Cells scanty.

A loopful of the exudate from a bronchiole was planted on blood agar slopes and plates which were examined after incubation for 24 hours at 37°C.

It will be noted that the B. influenzæ was found frequently in the exudate from the bronchioles. Examination was made by cultivation in 50 cases and the B. influenzæ was found to be present in 22 cases. The bacteriological findings were controlled by microscopical examination in each case. The small size of the B. influenzæ serves to distinguish it readily from other organisms. The pneumococcus was found 28 times out of 50 cases examined. Streptococci were not observed on microscopical or cultural examinations. Microscopical examination showed the presence of B. influenzæ in 36 cases out of 45 examined and the pneumococcus 42 times out of 45. Hence in the bronchioles, we had to deal with the B. influenzæ and pneumococcus. The examination of the cellular elements in the exudate from the bronchioles showed that they were composed mainly of polynuclear leucocytes. Phagocytosis was only occasionally observed; as a rule, the organisms were extra-cellular in distribution. Wolbach (3) found the

B. influenzæ in 23 cases out of 28 by cultural and histological methods combined, and in 14 of these in pure culture. Opie and his colleagues(16) demonstrated the B. influenzæ, 'with few, if any, exceptions in the bronchi of those who have had the purulent bronchitis which follows influenza.'

It is interesting to note that the B. influenzæ was cultivated 13 times out of 43 cases examined from the *bronchial glands*, and the pneumococcus 18 times out of 42. Microscopical examination of smears from the cut surface of the bronchial glands showed the presence of B. influenzæ in nine cases out of 34 examined, and the pneumococcus in 10 cases out of 34. As has been already shown, the bronchial glands were definitely enlarged in many cases and on section were seen to b acutely congested.

TABLE No. 29.

The following Table shows the result of the examination by cultivation of the bile in cases of Influenza.

Case No.	Duration of illness.	Presence or absence of organisms in the bile.
10	9 days	0
11	13 ,,	0
12	20 ,,	0
13	16 ,,	Sterile
14	13 ,,	,,
15	20 ,,	,,
16	16 ,,	,,
17	8 ,,	,,
18	27 ,,	,,
19	9 ,,	+ (B. coli)
20	....	Sterile
21	11 days	,,
22	9 ,,	,,
23	17 ,,	0
24	13 ,,	0
25	13 ,,	Sterile
26	....	0
27	6 days	Sterile
28	8 ,,	,,
29	....	0
31	4 days	0
33	5 ,,	0
34	....	0
35	....	0
36	....	Sterile
37	15 days	0
38	13 ,,	0
39	19 ,,	Sterile
41	9 ,,	,,

Symbols.

+ = Present. 0 = Not examined.

TABLE No. 29.—*Concld.*

Case No.	Duration of illness.	Presence or absence of organisms in the bile.
42	....	Sterile
43	7 days	0
44	7 ,,	Sterile
46	10 ,,	,,
47	8 ,,	,,
48	7 ,,	,,
49	10 ,,	,,
51	11 ,,	,,
52	12 ,,	,,
53	10 ,,	,,
54	10 ,,	+ (Staphylococci)
55	10 ,,	Sterile
56	11 ,,	,,
57	14 ,,	+ (Staphylococci)
58	15 ,,	0
59	11 ,,	+ (Staphylococci)
60	12 ,,	Sterile
61	13 ,,	,,
62	16 ,,	0
63	....	Sterile
64	13 days	0
65	5 ,,	Sterile
66	11 ,,	,,
67	10 ,,	0
68	18 ,,	Sterile

Symbols.

+ = Present. 0 = Not examined.

As will be seen from the above table, the results of the cultural examination of the bile showed that in most cases it was sterile. In a few cases staphylococci were cultivated, and on one occasion B. coli. After the culture was made, the gall bladder was examined, and in none of the cases was any definite pathological change observed, naked eye.

TABLE No. 30.

The following Table shows the result of the microscopical and cultural examination of the kidneys in cases of Influenzæ.

Case No.	Duration of illness.	KIDNEYS.			
		MICROSCOPICAL.		CULTURAL.	
		B. Influe.	Pneumococcus.	B. Influe.	Pneumococcus.
10	9 days	0	0	0	0
11	13 ,,	0	0	0	0
12	20 ,,	0	0	0	0

Symbol.

0 = Not examined.

TABLE No. 30.—*Concld.*

Case No.	Duration of illness.	KIDNEYS.			
		MICROSCOPICAL.		CULTURAL.	
		B. Influe.	Pneumococcus.	B. Influe.	Pneumococcus.
13	16 days	0	0	0	0
14	13 ,,	0	0	Sterile	Sterile
15	20 ,,	–	–	0	0
16	16 ,,	–	–	Sterile	Sterile
17	8 ,,	–	–	,,	,,
18	27 ,,	–	–	,,	,,
19	9 ,,	–	–	,,	,,
20	....	–	–	,,	,,
21	11 days	–	–	,,	,,
22	9 ,,	–	–	–	–(cocci)
23	17 ,,	0	0	0	0
24	13 ,,	0	0	0	0
25	13 ,,	–	–	Sterile	Sterile
26	....	0	0	0	0
27	6 days	–	–	Sterile	Sterile
28	8 ,,	–	–	,,	,,
29	....	0	0	0	0
31	4 days	–	–	0	0
33	5 ,,	0	0	0	0
34	....	0	0	0	0
35	....	0	0	0	0
36	....	–	–	Sterile	Sterile
37	15 days	–	–	,,	,,
38	13 ,,	0	0	,,	,,
39	19 ,,	–	–	,,	,,
41	9 ,,	–	–	,,	,,
42	....	0	0	,,	,,
43	7 days	0	0	,,	,,
44	7 ,,	0	0	,,	,,
46	10 ,,	–	–	,,	,,
47	8 ,,	0	0	–	– (Staphylococci)
48	7 ,,	0	0	Sterile	Sterile
49	10 ,,	0	0	–	– (Staphylococci)
51	11 ,,	–	–	Sterile	Sterile
52	12 ,,	0	0	,,	,,
53	10 ,,	0	0	0	0
54	10 ,,	–	–	Sterile	Sterile
55	10 ,,	–	–	,,	,,
56	11 ,,	–	–	,,	,,
57	14 ,,	–	–	,,	,,
58	15 ,,	–	–	Sterile	Sterile
59	11 ,,	–	–	,,	,,
60	12 ,,	–	–	,,	,,
61	13 ,,	0	0	0	0
62	16 ,,	0	0	0	0
63	....	–	–	Sterile	,,
64	13 days	–	–	,,	Sterile
65	5 ,,	–	–	,,	,,
66	11 ,,	–	–	,,	,,
67	10 ,,	–	–	,,	,,
68	18 ,,	–	–	,,	,,

Symbols.

0 = Not examined. – = Absent.

+ = Present.

At one time it was thought that the B. influenzæ escaped by the kidneys. The results of our bacteriological examination of the kidneys show that organisms could be cultured very rarely, and the B. influenzæ was never observed either on microscopical or cultural examination. The pneumococcus was found in one case. The results are interesting as showing that the pathological changes in the kidney are not brought about by the organisms themselves, but possibly by toxins.

TABLE No. 31.

The following Table shows the duration of illness, the result of the microscopical and cultural examination of the Cerebro-spinal Fluid removed post-mortem *from cases of Influenza.*

Case No.	Duration of illness.	CEREBRO-SPINAL FLUID.				
		MICROSCOPICAL.			CULTURAL.	
		Cells.	B Influe.	Pneu.	B. Influe.	Pneumococcus.
9	....	0	0	0	+	—
10	9 days	0	0	0	—	—
11	13 ,,	0	0	0	—	—
12	20 ,,	0	0	0	0	0
13	16 ,,	0	0	0	—	—
14	13 ,,	0	0	0	+	—
15	20 ,,	0	0	0	—	—
16	16 ,,	0	0	0	0	0
17	8 ,,	0	0	0	—	—
18	27 ,,	0	0	0	0	0
19	9 ,,	0	0	0	—	+
20	....	0	0	0	—	—
21	11 days	0	0	0	—	—
22	9 ,,	N—	—	+	—	—
23	17 ,,	0	0	0	—	+
24	13 ,,	0	0	0	—	—
25	13 ,,	0	0	0	—	—
26	....	0	0	0	0	0
27	6 days	0	0	0	—	—
28	8 ,,	N—	—	—	—	—
29	....	N+ (Pus base of brain)	+	+	—	—
31	4 days	N—	—	—	0	0

Symbols.

N — = Cells diminished in number. 0 = Not examined.
N + = Cells increased in number. + = Present.
— = Absent.

TABLE No. 31.—*Concld.*

Case No.	Duration of illness.	CEREBRO-SPINAL FLUID.				
		MICROSCOPICAL.			CULTURAL.	
		Cells.	B. Influe.	Pneu.	B. Influe.	Pneumococcus.
32	....	N+ (Intraven. fluid purulent)	—	+	—	—
33	5 days	N+ (Pus base of brain)	—	—	—	—
34	....	N	—	—	—	—
35	....	N+ (Pus base of brain)	—	—	—	—
36	....	N+	—	—	—	—
37	15 days	N+	—	—	—	—
38	13 ,,	0	0	0	—	— (Staphylococci)
39	19 ,,	N	—	—	—	—
41	9 ,,	N	—	—	—	— (,,)
42	....	N	—	—	—	— (,,)
43	7 days	N	—	—	—	— (,,
44	7 ,,	N	—	—	—	—
46	10 ,,	N	—	—	—	—
47	8 ,,	N	—	—	—	—
48	7 ,,	N	—	—	—	—
49	10 ,,	N	—	—	—	—
51	11 ,,	N	—	—	—	—
52	12 ,,	N	—	—	—	—
53	10 ,,	N+	—	—	—	—
54	10 ,,	N	—	—	—	—
55	10 ,,	N	—	—	—	—
56	11 ,,	N	—	—	—	—
57	14 ,,	N+	+	+	—	+
58	15 ,,	N	—	—	—	—
59	11 ,,	0	0	0	—	—
60	12 ,,	N	—	—	—	—
61	13 ,,	N	—	—	—	—
62	16 ,,	N+	—	+	—	+
63	....	N	—	—	—	—
64	13 days	N+	—	—	—	—
65	5 ,,	N+	—	—	—	—
66	11 ,,	N+	—	+	—	+
67	10 ,,	N	—	—	—	—
68	18 ,,	N	—	—	—	—

Symbols.

N = Cells normal.
N + = Cells increased in number.
0 = Not examined.
+ = Present.
— = Absent.

The results of the bacteriological examinations of the cerebro-spinal fluid have been discussed in a previous paper (4). The above table

contains the further results, additional cases having been examined since publication.

It will be noted that the microscopical examination showed that the number of the cells in the cerebro-spinal fluid was increased in 11 cases out of 36 examined, and pus was actually present in four cases; B. influenzæ was seen in two cases out of 36 examined, and the pneumococcus in six out of 36 examined. The B. influenzæ was cultivated on two occasions out of 51 cases from which cultures were made. The pneumococcus was recovered five times out of 51 cases examined culturally. Lyon ([10]) obtained cultures of pneumococci and staphylococci from the pia-arachnoid in one of the cases. Wolbach ([3]) records cerebral abscesses in one of his cases.

Examinations of the Blood in Influenza cases.

Widal reaction in Convalescence.

Table No. 32.

Widal reaction of Blood in cases of Influenza.

Case No.	Date of onset of illness.	Date of taking blood.	B. influenzæ. Widal reaction. 1-20 : 1-40 : 1-80			Presence or absence of B. influenzæ in culture from sputum.
5	15- 9-18	10-10-18	−	−	−	+
18	22- 9-18	18-11-18	−	−	−	+
40	30- 9-18	18-11-18	±	−	−	+
42	1-10-18	18-11-18	−	−	−	−
43	..	..	−	−	−	+
46	3-10-18	..	+	±	−	+
51	4-10-18	..	−	−	−	+
52	..	11-11-18	−	−	−	+
53	..	12-11-18	±	±	−	+
56	5-10-18	11-11-18	+	±	−	+
57	..	..	±	−	−	+
58	..	..	−	−	−	+
59	..	13-11-18	+	+	±	+
65	7-10-18	12-11-18	−	−	−	+
70	8-10-18	..	−	−	−	+
76	9-10-18	11-11-18	−	−	−	+
79	..	..	−	−	−	+
80	..	12-11-18	±	−	−	+
81	10-10-18	11-11-18	+	±	−	+
82	..	13-11-18	−	−	−	+
88	12-10-18	11-11-18	−	−	−	+
93	14-10-18	12-11-18	−	−	−	+

Symbols.

+ = Reaction complete. ± = Reaction incomplete. − = Negative.

TABLE No. 32.—*Concld.*

Case No.	Date of onset of illness.	Date of taking blood.	B. INFLUENZÆ. WIDAL REACTION. 1–20 : 1–40 : 1–80.			Presence or absence of B. influenzæ in culture from sputum.
97	..	..	±	-	-	+
98	..	.	-	-	-	+
99	..	..	±	-	-	-
100	15–10–18	11–11–18	-	-		+
103	..	13–11–18		-	-	+
110	17–10–18	12–11–18	-	-	-	+
111	20–10–18	13–11–18	-	-		+
113	..	12–11–18	-			+
114	..	13–11–18	-	-	-	+
115	21–10–18	12–11–18	-	-	-	+
117	..	..	-	±	-	+

Symbols.

\+ = Reaction complete. ± = Reaction incomplete. − = Negative.

In the above series the B. influenzæ had been recovered from the sputum in all the cases whose blood was examined for the presence or absence of agglutinins to B. influenzæ. The serum was put up in Wright's tubes with an emulsion of B. influenzæ in the usual manner and kept at 37°C. for 1 hour, and the results read. It will be seen that agglutinins for B. influenzæ develop, but in rather small amounts. Gay and Harris ([17]) have shown that better results are obtained by incubating at 55°C. for 3—6 hours. They found by this method that :—'Nearly 90 per cent of the acute cases of influenza gave positive agglutination reactions in a dilution of from 1 : 10 to 1 : 160. Recovered cases in 38·5 per cent apparently irrespective of the time after recovery.' They say that very few normal sera and sera of other acute febrile conditions were available as controls on the positive reactions.

Fildes and MacIntosh ([18]) state :—'Although, therefore, there is no satisfactory evidence that B. influenzæ is pathogenic to man, so far as the inoculation of cultures goes, nevertheless the constant occurrence of anti-bodies to this bacillus in the serum of patients makes it clear that it has a definite pathogenic action which cannot, however, be reproduced. The presence of the anti-bodies may be taken as evidence that the bacillus is a pathogenic bacillus, such as might be the cause of influenza, but not that the characteristic lesions of this disease are due to this pathogenic bacillus.'

BLOOD COUNTS IN CASES OF INFLUENZA.

TABLE No. 33.

Non-fatal cases.

Case No.	Date of admission.	Date of observation	Total W.B.C.	P.N.	L.M.	S.M.	E.
5	15-9-18	16- 9-18	8,424	83	7	10	0
		27- 9-18	8,592	84	9	7	0
19	22-9-18	25- 9-18	5,469	60	18	21	1
		30- 9-18	13,750	78	8	13	1
35	28-9-18	28- 9-18	29,942	83	8	9	0
		4-10-18	9,431	77	8	14	1
36	..	28- 9-18	6,250	72	9	18	1
40	30-9-18	30- 9-18	19,188	71	10	15	4
42	1-10-18	1-10-18	13,437	83	12	5	0
43	1-10-18	1-10-18	6,094	58	23	29	0
46	3-10-18	3-10-18	5,000	74	11	15	0
47	3-10-18	3-10-18	5,625	63	14	23	0
		4-10-18	10,000	60	12	28	0
49	4-10-18	4-10-18	9,062	76	8	16	0
51	4-10-18	5-10-18	2,655	62	10	28	0
52	..	5-10-18	6,250	69	14	17	0
53	..	5-10-18	3,593	76	8	16	0
56	5-10-18	5-10-18	7,969	64	13	23	0
62	7-10-18	8-10-18	4,375	77	5	18	0
63	..	..	10,000	62	10	28	0
64	..	..	6,718	66	9	25	0
73	8-10-18	9-10-18	11,306	78	9	13	0
75	..	..	4,375	67	15	18	0
		12-10-18	4,844	75	7	18	0
		19-10-18	39,780	..	..	..	..
93	13-10-18	15-10-18	4,844	71	9	20	0
115	20-10-18	20-10-18	8,272	72	9	19	0

Total and differential counts of the leucocytes were made in a series of non-fatal cases. In cases in which the lungs were markedly involved the total count was higher than in those in which the signs were not so marked. In some of the cases there was a relative increase of the polynuclear leucocytes. Lyon ([10]) found the average leucocyte count in fatal cases 5,537 ; with extremes of 2,675 to 11,900.

EXPERIMENTAL INOCULATION OF BLOOD FROM CASES OF INFLUENZA.

The blood from severe cases of influenzal pneumonia was inoculated into guinea-pigs.

Experiment 1. *15th February* 1919.—2·5 c.c. of blood taken from the vein at bend of elbow was injected at once into the peritoneal cavity of a guinea-pig.

16*th February* 1919.—Marked drop in temperature of the animal.

17*th February* 1919.—Two days after inoculation the animal died.

Post-mortem examination showed changes in the alimentary canal similar to those described in man. A pure culture of pneumococcus was obtained from the heart-blood, peritoneal cavity and liver. Microscopical examination showed the presence of pneumococcus in all the other organs.

It is interesting to note that the patient from whom the blood was taken ultimately recovered, although severely ill. He had a pneumococcic septicæmia.

Experiment 2. 14*th February* 1919.—2·5 c.c. of blood from the vein at the bend of elbow was injected at once into the peritoneal cavity of a guinea-pig.

On 16*th* and 17*th February* 1920, the temperature of the animal was low. It rose on the 18*th February* 1919, but fell on 19*th February* 1920. The animal was killed on 21*st February* 1919.

Post-mortem examination.—In the alimentary tract the jejunum showed marked congestion of the mucous membrane. The pleural surfaces of the lungs were also congested.

Bacteriological examination of the heart-blood, peritoneal fluid and liver showed that they were sterile. No organisms could be seen on microscopical examination of the organs.

The patient in this case was also severely ill, but recovered.

In this case, although the animal reacted to the injection, a pneumococcic invasion of the tissues did not occur in this case.

Other experiments.—Several other guinea-pigs were injected with blood from cases, but, although a drop in temperature followed the injection, the animals recovered.

Remarks.—The results of the experiments are interesting. They prove that there was a pneumococcic infection of the blood of the patients. The animals injected showed a characteristic drop of temperature following inoculation. The lesions found *post-mortem* in the alimentary tract were noteworthy, specially in view of the fact that similar changes were found in the alimentary tract of influenza cases as already mentioned. MacCallum (1) states :—'The question of the existence of a streptococcal septicæmia is much discussed and there are differences of opinion on the point. We have made in all about 100 blood cultures from the circulating blood taken from the arm at various times before death with the usual technique of adding the blood to sterile

broth, etc., but none of these cultures gave a positive result except when they were taken just before death. Even then, there were very few positive results and the contrast between this organism and the pneumococcus is made more striking by the fact that in the cases in which lobar pneumonia is found combined with interstitial broncho-pneumonia the pneumococcus has been isolated from the circulating blood, but not the streptococcus.'

Fildes and MacIntosh ([18]), in concluding an interesting recent review on the ætiology of influenza, sum up the position as regards B. influenzæ as follows :—' Thus, we have a characteristic living organism which is present in the lesions of influenza and has a pathogenic action for man. Pure cultures of this bacillus are capable of producing in animals a condition which has important points in common with influenza in man, and it is only the indefinite nature of the essential lesions of influenza which causes hesitation in accepting the experimental disease in animals as the natural disease in man.

If this relationship is accepted, then the criteria by which a bacterium should be judged the cause of a disease are fulfilled.'

All the experimental evidence so far leaves unexplained the most essential feature of the disease, namely, its sudden occurrence in 1918 in epidemic form of unparalleled severity attacking every country in the world simultaneously.

Nature, in an editorial article, states :—' Medicine has to face the fact that, for practical purposes, it knows nothing about the cause of measles, scarlet fever, mumps, influenza, rheumatic fever, cancer or other forms of malignancy.'

Dr. Winternitz of Yale, who has been working at the pathology of poisoning by noxious gases, chlorine, mustard gas, phosgene and organic arsenic preparations, informed me during an interesting visit to his laboratory that the investigations had thrown light on the influenza problem. Chlorine and mustard gas completely kill the epithelium of the trachea and bronchi and permit of secondary bacterial invasion. Further, he informed me that in infecting animals intratracheally with pneumococcus the reaction of the tissue is very important in determining local infection. Thus, a culture of pneumococcus which produces acid introduced into the trachea will start an infection. As regards the pneumococcus there are three factors at work :—(1) Toxic. (2) Mechanical. (3) Metabolic, acid or alkaline reaction. A 1 per cent solution of HCl introduced into the trachea produces rapid death, a

0·5 per cent solution HCl produces a definite reaction in the lungs. There is individual variation in animals.

Conclusions.

1. It will be seen that associated with measles characteristic pathological changes are seen in the respiratory system described by MacCallum as interstitial broncho-pneumonia and the latter is due to a hæmolytic streptococcus.

2. The experience in India showed that the pathological lesions were those associated with influenza and in the respiratory system it took the form of an acute bronchiolitis without serious involvement of the pleura, such as empyema, also other serous membranes, *e.g.*, pericardium and peritoneum, were not attacked; the organisms associated with the lesions were B. influenzæ and the pneumococcus, the hæmolytic streptococcus did not play a part in the causation of the disease.

I am indebted to Captain G. C. Maitra, I.M.S., for assistance in the bacteriological portion of this investigation.

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CHEMICAL COMPOSITION OF NIM OR MARGOSA OIL.

BY

K. K. CHATTERJI, F.R.C.S.I.,

Surgeon and Dermatologist, Campbell Hospital, Calcutta ;

AND

R. N. SEN, M.A., M.SC., F.C.S.,

Professor of Chemistry, Presidency College, Calcutta.

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In the Pharmacographia Indica, Vol. I, by Dymock, Warden and Hooper, it is stated that Nim oil contains :—

(1) A small quantity of a natural resin containing sulphur and possessing a very bitter taste and also a marked odour resembling that of the oil, the total unsaponifiable matter amounting to 1·60 per cent ;

(2) A small quantity of a substance with the properties of an alkaloid ;

(3) Small quantities of two other resins with marked acid properties ;

(4) Glycerides of fatty acids—the soluble fatty acids amounting to 3·519 per cent, the insoluble amounting to 89 128 per cent ; butyric acid and a trace of valeric acid being detected as volatile acids. The same authors also mention that the neutralisation value of the mixed insoluble fatty acids was found to be 138·8 m. grams NaOH or 194·32 m. grams KOH, but no attempt at separating the insoluble fatty acids was made (Pharmacographia Indica, Vol. I).

Nim oil was also examined by Lewkowitsch and the following physical and chemical characteristics were determined by him :—

Sp. Gravity at 16° C. (water 1)	0·9142
Saponification value	196·9
Iodine value	69·6
Reichert Meissel value	1·1

Refractive Index (Butyro refractometer at 40° C.) degrees 52.

Titer test of the insoluble mixed fatty acids 42° C. (Chem. Tech. and analysis of Oils, Fats, Waxes by Lewkowitsch.)

The preliminary examination of a commercial sample of Nim oil gave the following results :—

Sp. Gravity at 30·7° C.	0·9149
Saponification value	197·92
Acid value	22·146
Iodine value	69·23
Unsaponifiable matter	1·91 per cent
Total acids	93·22 per cent

of which about 88 per cent constitutes insoluble acids.

Sulphur was detected in the unsaponifiable matter.

The mixture of insoluble fatty acids of Nim oil was then prepared by standard methods usually adopted for the purpose and then purified by redissolving in hot solutions of caustic alkalies and filtering, followed by reprecipitation with dilute H_2SO_4 and repeated washing with hot water. The acid was then melted on the water-bath, separated from water and finally dried. The acid thus prepared is semi-solid at ordinary temperatures in summer, becoming harder in winter. It melts completely at 40°–41° C. It is of a slightly brownish colour and has a more or less bitter taste and the characteristic odour of the oil. It dissolves completely in hot alcohol and other solvents of fatty acids. It gives a slight indication of Lieberman-Storch reaction for resin and hence it appears that the colour and taste of the acid are probably due to the remaining traces of resinous matter originally present in the oil. When the acid is freed from unsaponifiable matter, it melts at 42° C. and its bitter taste practically disappears, but the brownish colour still persists. It therefore appears that the bitter taste of Nim oil is due to an unsaponifiable constituent of the oil, which is also slightly soluble in water. The neutralisation value of a sample of the mixed acid is 188·7 (with unsaponifiable matter) and 192·32 (when free from unsaponifiable matter) and its iodine value is found to be 69·35 (with unsaponifiable matter) and 68·11 (when free from unsaponifiable matter).

The approximate proportion of the liquid and the solid acids (insoluble in water) in Nim oil was determined by the Cusserow Varrentrap method with the following result : –

3·0248 grams of Nim oil gave 1·815 grams of liquid acid and ·8466 gram of solid acid.

Therefore, liquid acid = 60·003 per cent of the oil ;
and solid acid = 27·98 per cent of the oil.

The mixed insoluble acid obtained from the oil was also separated into solid acid (saturated) and liquid acid (unsaturated) by the lead-salt-ether process of Tortelli and Rugari.

The solid acid thus separated has the following characteristics :–

Neutralisation value = 19
Iodine value = 12
Melting point = 55·2° C.
Colour .. .. almost white.

The iodine value indicates the presence of unsaturated acids in a very small quantity. This solid acid, after being once recrystallised from alcohol, became much whiter and melted at 62° C. ; after second recrystallisation it was quite white and melted at 67° C. and after several recrystallisations, its melting point rose to 68°–69° C. Thus the solid acid (about 28 per cent of the oil) is proved to be stearic acid, which melts at 69° C. and has a neutralisation value of 197·5. The liquid acid separated from the mixed insoluble acid has the following characteristics :—

Neutralisation value = 185·89
Iodine value = 105–110
Sp. Gravity at 34·4° C. = ·8920

It is of a slightly yellowish colour with a characteristic smell. It is a liquid at ordinary temperatures and solidifies at about ·8° C. It partially answers to the elaidin test indicating the presence of some acids like oleic acid. On bromination, no insoluble bromide is formed from which it is inferred that it does not contain any acid less saturated (or more unsaturated) than linolic. The constituents of the liquid acid were next separated in the following manner :—A weighed quantity of the liquid acid was dissolved in alcohol and neutralised with alcoholic caustic potash solution using phenolphthalein as indicator. Then a little excess of KOH was added and the alcohol was distilled off. The residue was dissolved in hot water and treated with a boiling solution of barium chloride till the precipitation was complete. The precipitate was washed with water by decantation till free from chlorides and drained

by the filter pump as completely as possible. The precipitate in a slight moist condition was dissolved by boiling in a mixture of benzene and alcohol (100 c.c. : 10 c.c.). The clear solution was then cooled down to 6°–7° C. when a white crystalline product separated out. This was filtered by the pump. The residue (insoluble Ba-salt) was then redissolved in hot benzene and treated with hot dilute HCl to liberate the fatty acid (I) which was recovered in the usual manner and weighed. The filtrate from the insoluble Ba-salt was also treated with dilute HCl to liberate the fatty acid (S) corresponding to the Ba-salt soluble in cold benzene, which was also recovered and weighed.

3·233 grams of liquid acid gave 1·0574 grams of acid (S) and 1·5595 grams of acid (I). Loss = ·6161 gram.

The proportion of the acid (S) to the acid (I) is thus found to be about 2 : 3.

The acid (I) has the following characteristics :—

Neutralisation value = 199·7
Iodine value = 90·9
Solidifying point = 3·5° C.

The Ba-salt of acid (I) purified by recrystallisation from hot benzene solution gave on analysis :—

Ba = 19·5 per cent
Ba-oleate [Ba (C_{18} H_{33} O_2)$_2$] requires Ba = 19·6 per cent

It also readily answers to the elaidin test.

The acid (I) is thus found to be oleic acid.

The acid (S) has the following characteristics :—

Colour—slightly yellowish.
Neutralisation value = 167
Iodine value = 94–95 (when separated from the mixed acids in the manner described above) and = 131–132 (when the acid is obtained directly from the oil with special precautions to prevent oxidation in the manner described below).
Solidifying point = —10° to —11° C.
Sp. Gravity at 34·4° C. = ·8693.

The Barium-salt of acid (S) prepared in ordinary aqueous solution gave Ba = 11·92 per cent ;

and when prepared in the presence of sufficient alcohol the Barium-salt gave Ba = 17·27 per cent.

It does not answer to the elaidin test.

Now the fatty acid having the formula $C_{22}H_{40}O_2$ should have the following theoretical constants :—

Neutralisation value = 166·7
Iodine value = 151

The normal Barium-salt Ba $(C_{22}N_{39}O_2)_2$ should contain
Ba = 16·98 per cent,
and the acid salt Ba $(C_{22}H_{39}O_2)_2$. $C_{22}H_{40}O_2$ should contain
Ba = 11·98 per cent.

It appears, therefore, that the acid (S) is identical with the unsaturated fatty acid having the formula $C_{22}H_{40}O_2$ belonging to the linolic acid series.

Only the iodine value of the acid (S) is much below the theoretical number; this is supposed to be due to unavoidable oxidation of the acid by air in the long process of its separation, since higher iodine values are obtained as the oxidation is diminished.

The higher iodine value (132) was found in the acid prepared in the following manner, with ordinary precautions to prevent oxidation. About 20 grams of Nim oil were taken and saponified with 15 c.c. of 50 per cent. KOH solution and 15 c.c. of alcohol. After the saponification, the excess of KOH was neutralised with acetic acid and the solution was then made slightly alkaline with a little KOH solution. This solution was then slowly poured into a flask containing about 300 c.c. of a moderately strong boiling solution of Barium chloride while the flask was shaken constantly. The flask was then cooled to the ordinary temperature and the clear water poured off. The Barium-salt formed on the sides of the flask was washed with water till free from chlorides and pressed with dry filter paper to remove the adhering water. The slightly moist salt was then dissolved in about 250 c.c. of hot benzene-alcohol mixture (100 c.c. of benzene : 10 c.c. of alcohol), and the solution was cooled down to 6°–7° C. and quickly filtered under the pump.

The filtrate (which should remain clear at 6°–7° C.) was then taken into a separating funnel and treated with 150 c.c. of dilute HCl (1 : 2) and the aqueous layer was separated. The operation was repeated with 100 c.c. of HCl (30 : 70) and the aqueous layer was again removed. The solution of the liberated fatty acid in benzene was washed with water till free from HCl and filtered into a 250 c.c. measuring flask and made up to 250 c.c. with pure benzene. This solution was used immediately in the determination of the characteristics of the acid; and the acid itself was obtained by evaporating off the solvent in an atmosphere of CO_2.

In connection with the Barium compound, it was found that the normal salt of the acid (S) is obtained with difficulty only from the alcoholic solution of the alkali salt of the acid (S), while the acid salt is

easily formed in aqueous solutions. In the case of the acid (I), *i.e.*, oleic acid, the normal salt is easily obtained from ordinary aqueous solutions. These observations are supported by the recent researches on the soap solutions and the salts of fatty acids.

Thus it is proved that the liquid acid of Nim oil separated by the lead-salt-ether process is a mixture of the two different liquid acids—(1) oleic acid of which the Ba-salt is soluble in hot but insoluble in cold benzene-alcohol mixture and (2) the acid (S) having the formula $C_{22}H_{40}O_2$ and belonging to the linolic acid series, of which the Ba-salt is very soluble in cold benzene-alcohol mixture. This conclusion also agrees well with the neutralisation value and the iodine value of the mixed liquid acid actually found by experiment.

Thus we have :	N. V.	I. V.
Acid oleic ($C_{18}H_{34}O_2$)	= 198·5	90
Acid (S) ($C_{22}H_{40}O_2$)	= 166·6	151
Mixed liquid acid	= 185·9	105 –110.

Let x and y be the percentages of oleic acid and acid (S) in the mixture, then we have,

$$x + y = 100$$

$$\frac{198{\cdot}5\ x}{100} + \frac{166{\cdot}6y}{100} = 185{\cdot}9, \quad \therefore \begin{matrix} x = 60{\cdot}5 \\ y = 39{\cdot}5 \end{matrix}$$

i.e., acid (S) and oleic acid are present in the ratio of about 2 : 3 (as also found directly).

Hence the calculated iodine value

$$= \frac{90 \times 60{\cdot}5}{100} + \frac{151 \times 39{\cdot}5}{100} = 114$$

the iodine value determined by experiment being 105–110. Therefore the acid (S), which has been called Margosic acid, has been given the formula $C_{22}H_{40}O_2$ which has been confirmed by the estimation of Barium in its Barium-salt already mentioned. This acid (S) having the formula $C_{22}H_{40}O_2$ appears to be the characteristic acid of Nim oil or Margosa oil and may therefore be termed Margosic acid. It is not known to have been obtained before from any other oil. It belongs to the linolic acid series. On bromination, it gives a bromide which is not insoluble in acetic acid and ether and which is also fairly soluble in petroleum ether. It does not answer to the elaidin test.

Thus it is proved that Nim oil contains about 88 per cent of insoluble fatty acids consisting of (in round numbers)

Stearic acid = 28 per cent
Oleic acid = 36 per cent
Margosic acid = 24 per cent

The iodine value of the oil calculated from the above composition

$$= \frac{24 \times 151}{100} + \frac{36 \times 90}{100} + \frac{28 \times 0}{100}$$ (assuming the remaining 12 per cent to have no iodine value)

$$= 36{\cdot}24 + 32{\cdot}4 = 68{\cdot}64 \text{ against } 69{\cdot}23;$$

the calculated neutralisation value of the mixed insoluble acid

$$= \frac{197{\cdot}5 \times 28 + 198{\cdot}5 \times 36 + 166{\cdot}6 \times 24}{88}$$

$= 189{\cdot}5$ against $192{\cdot}3$ actually found

and the calculated iodine value of the mixed insoluble acid

$$(24 \times 151 + 36 \times 90) \times \frac{100}{88} = 78 \text{ against } 68{\cdot}11$$ actually found (the difference being presumably due to unavoidable oxidation during the separation of the mixed acid from the oil).

THE WEIGHT AT BIRTH OF INFANTS IN INDIA.

BY

DAGMAR F. CURJEL, M.D. (Glasg.),

Women's Medical Service, India.

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THE conclusions reached in the report issued by the Medical Research Committee(1) regarding the relative importance of pre-natal and post-natal conditions as causes of Infant Mortality are :—That chances of survival differ but little at birth in different social classes, but as the child grows older the mortality in unfavourably situated classes becomes progressively higher.

It would appear that Nature favours the child at the expense of the mother, but mortality rates are not the sole index of vigor, and as yet much comparative information regarding physical development is not available.

In Glasgow, Dr. Kerr Love found that the children of the poorest mothers weigh on an average 7·1 lbs. at birth, the weight of a healthy infant being 7 lbs. (Evidence given before the Royal Commission on Venereal Diseases.)

In view of the very large number of infants who die before the end of the first year in India, the following enquiry was undertaken with the object of determining the condition of development and general nutrition, as shown by weight, of the normal Indian infant at birth. Weight was taken as a useful index of development, but in the present state of knowledge it is not possible to estimate the full extent to which unfavourable pre-natal conditions may have depressed the vitality of the child.

A normal infant was considered for the purposes of the enquiry to be a full-term birth, the presentation being either vertex or breech, where the labour had terminated naturally and spontaneously.

The weights at birth of 1,849 normal infants were collected by medical women in different parts of India (Bengal, Bihar and Orissa, Central India, Central Provinces, Delhi, Gujerat, Madras Presidency, United Provinces). The mothers were drawn from all classes of the community; the results are grouped under the headings Mussalman, Hindu, Out-Caste, and Indian Christian.

For comparison, the weights at birth of 289 normal infants born in India of other than Indian parentage (European, Anglo-Indian, Parsee), were also collected.

The method of weighing was to record the weight to the nearest quarter of a pound.

WEIGHT AT BIRTH OF INFANTS IN INDIA. NORMAL CASES.

			Weight in pounds.								
Indian	1,849 cases	Average weight in lbs. 6·48	3–4 44	4–5 139	5–6 382	6–7 708	7–8 424	8–9 127	9–10 22	10–11 3	11–12 ..
Mussalman ..	305 cases	Average weight in lbs. 6·44	10	34	54	102	78	25	2	..	..
Hindu ..	1,018 cases	Average weight in lbs. 6·45	26	84	207	398	222	63	16	2	..
Out-Caste ..	63 cases	Average weight in lbs. 6·9	.	..	12	22	19	10	..	..	..
Indian Christian ..	463 cases	Average weight in lbs. 6·5	8	21	109	186	105	29	4	1	..
Non-Indians ..	289 cases	Average weight in lbs. 7·39	1	5	30	80	84	60	21	6	2
Parsee ..	88 cases	Average weight in lbs. 6·8	..	3	17	31	25	11	..	1	..
European and Anglo-Indian	201 cases	Average weight at birth lbs. 7·64	1	2	13	49	59	49	21	5	2

Conclusions.

1. Records of the weights at birth of 1,849 normal Indian infants, representing many different castes and classes, show that the Indian infant has reached at the time of birth the average weight of 6·5 lbs.

2. This average compares favourably with the weights at birth of infants of other parentage born in India. Parsee (88 cases), average 6·8 lbs. and European and Anglo-Indian (201 cases), average 7·64 lbs., the latter group representing a more heavily built race.

3. There is no very appreciable difference in development as shown by average weight at birth of normal infants, between Mussalmans (305 cases) 6·44 lbs., Hindus (1,018 cases) 6·45 lbs., and Indian Christians (463 cases) 6·5 lbs.

4. The earlier marriage age of the Indian mother (as compared with European races), does not adversely affect the development and general nutrition, as shown by the weight, of her child at birth.

5. Since the normal Indian infant has reached a fair condition of development and nutrition as shown by the average weight 6·5 lbs. at birth, it follows that the subsequent high mortality among such children is largely due to unfavourable post-natal conditions. These conditions are intimately connected with the environmental factors affecting the health of the community generally, and demand similar remedies.

I have to acknowledge with many thanks the assistance given by a large number of medical women in obtaining these weights.

Reference.

[1] Medical Research Committee Special Report Series No. 10, 'The Mortalities of Birth, Infancy, and Childhood.'

THE REPRODUCTIVE LIFE OF INDIAN WOMEN.

BY

DAGMAR F. CURJEL, M.D. (Glasg.),

Women's Medical Service, India.

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A KNOWLEDGE of the average age of the onset of puberty, its association with early marriage, and the average duration of menstrual life among Indian women, is desirable for the correct treatment of the various morbid gynæcological conditions associated with these ages.

I.—The Onset of Puberty.

The conclusions generally held regarding the age of onset of puberty among European races are that the average age of onset is 14 years, but the limit of normal puberty is much wider, ranging from 12 to 16 years; that probably climate is less important than race, but other factors, such as nutrition, environment and heredity, come into play, some retarding, others advancing, the process.

To obtain some knowledge regarding conditions among Indian women, personal enquiries were made by medical women in different parts of India (Bengal, Berar, Bihar and Orissa, Bombay Presidency, Central India, Central Provinces, Delhi, Gujerat, Hyderabad (Deccan), Madras Presidency, Punjab, United Provinces). The women questioned represented many different castes and races, and were drawn from all classes of the community.

The results have been grouped together for comparison under the headings Mussalman, Hindu, and Indian Christian, since the returns for any one province or caste were not sufficiently distinctive to merit separate tabulation.

Age of onset of puberty.

Mussalman. Average 13·64 years.	Hindu. Average 13·62 years.	Indian Christian. Average 13·69 years.
1 began at 10 years	1 at 9 years	2 at 11 years
12 „ „ 11 „	5 „ 10 „	15 „ 12 „
25 „ „ 12 „	16 „ 11 „	23 „ 13 „
37 „ „ 13 „	49 „ 12 „	25 „ 14 „
29 „ „ 14 „	64 „ 13 „	5 „ 15 „
15 „ „ 15 „	55 „ 14 „	7 „ 16 „
10 „ „ 16 „	44 „ 15 „	1 „ 17 „
7 „ „ 17 „	24 „ 16 „	3 „ 18 „
1 „ „ 18 „	5 „ 17 „	
1 „ „ 19 „	1 „ 18 „	
2 „ „ 20 „	1 „ 19 „	
....	3 „ 20 „	
140	268	81

Results.—Among 489 Indian women, the average age of onset of puberty was 13·63 years.

The value of figures obtained by questioning women of perhaps limited education and inexact memory may be questioned, therefore figures were obtained for a group of well-educated women, college students (unmarried), from different parts of India.

College students, age of onset of puberty. Average 13·87 years.

1	began	at	10	years
2	„	„	11	„
5	„	„	12	„
15	„	„	13	„
17	„	„	14	„
9	„	„	15	„
4	„	„	16	„
1	„	„	17	„
2	„	„	18	„
56				

Among 56 well-educated, unmarried Indian women, the average age of onset of puberty was stated to be 13·87 years.

During the course of an enquiry on Osteomalacia, Scott (1) records that among 81 Indian women, where menstruation was normal, the average age of puberty (catamenia) was 14·07 years.

The age of the onset of puberty (catamenia) among Indian women appears to lie within the limits for European races.

II.—Early Marriage in relation to the Onset of Puberty.

In view of the earlier age at marriage among Indian women, it was decided to consider the age of the wife at marriage in relation to the onset of puberty (catamenia).

Since the marriage contract may take place at a very early age, the date asked was that of the wife going to live in her father-in-law's house, and this date is considered as marriage for the purposes of this enquiry.

Age of wife at marriage.

Mussalman. Average 13·84 years.		Hindu. Average 12·91 years.		Indian Christian. Average 16·82 years.	
At 8 years	2	At 7 years	6	At 10 years	2
„ 9 „	4	„ 8 „	8	„ 11 „	1
„ 10 „	5	„ 9 „	7	„ 12 „	8
„ 11 „	11	„ 10 „	25	„ 13 „	3
„ 12 „	23	„ 11 „	26	„ 14 „	8
„ 13 „	15	„ 12 „	33	„ 15 „	5
„ 14 „	24	„ 13 „	52	„ 16 „	14
„ 15 „	26	„ 14 „	37	„ 17 „	9
„ 16 „	12	„ 15 „	31	„ 18 „	11
„ 17 „	5	„ 16 „	24	„ 19 „	4
„ 18 „	4	„ 17 „	6	„ 20 „	5
„ 19 „	2	„ 18 „	4	„ 21 „	3
„ 20 „	3	„ 19 „	0	„ 22 „	2
„ 21 „	0	„ 20 „	1	„ 23 „	1
„ 22 „	2	„ 21 „	0	„ 24 „	2
		„ 22 „	0	„ 25 „	1
		„ 23 „	1	„ 26 „	0
		„ 24 „	1	„ 27 „	1
				„ 28 „	0
				„ 29 „	1
	138		262		81

Results.—Of the 489 Indian women referred to in I the answer of 8 are not recorded; of the remaining 481 women, the average age of marriage was 13·83 years.

In the 81 cases (chiefly Mussalman and Hindu women) collected by Scott(2), the average age of the onset of puberty, 14·07 years, was found to be closely allied to the age of marriage (bringing home of bride to father-in-law's house), 13·2 years. It would appear, however, that the age of the wife at marriage exerts but little influence on the onset of puberty, since, in the cases here recorded, it is shown that although the average age of the wife at marriage among Indian Christians is decidedly higher (16·82 years), than among Mussalmans (13·84 years), or Hindus (12·91 years), yet the average age of onset of puberty among the women in these three groups remains practically equal (13·6 years), and is comparable with the average age of onset among a group of unmarried women students (13·87 years).

Harvey (3) records the average age at marriage of Brahmin females (246 marriages) as 11·37 years. This somewhat lower average probably results from all the observations referring to one caste only, the average for Hindus (12·91 years) given above being the result obtained from observations among a large number of different castes.

The age of the wife at marriage does not exert any very appreciable influence on the age of the onset of puberty among Indian women.

III.—The Duration of Menstrual (Reproductive) Life.

The average duration of the reproductive or menstrual life for European countries is placed at about 30 years, but its cessation (menopause) may vary in its occurrence as widely as its onset (catamenia or puberty). The average climacteric occurs between the 40th and the 45th year, but normal limits are much wider, ranging between the 40th and 55th year. Climate and other accidental factors are not considered to exert the same influence on the cessation of the reproductive function as on its commencement.

Women are often uncertain as to the exact date of the cessation of a function which may terminate gradually, and the following figures for 74 cases collected by medical women (at the same time as the data referred to in I and II) can therefore only be considered as approximately correct.

Duration of menstrual life. Average 32·14 years.

1	case	15	years' duration.
2	cases	16	,, ,,
3	,,	17	,, ,,
1	case	21	,, ,,
1	,,	23	,, ,,
4	cases	24	,, ,,
2	,,	25	,, ,,
2	,,	26	,, ,,
5	,,	27	,, ,,
3	,,	28	,, ,,
6	,,	29	,, ,,
1	case	30	,, ,,
5	cases	31	,, ,,
4	,,	32	,, ,,
8	,,	33	,, ,,
5	,,	34	,, ,,
1	case	35	,, ,,
6	cases	36	,, ,,
6	,,	37	,, ,,
1	case	38	,, ,,
2	cases	39	,, ,,
1	case	40	,, ,,
2	cases	42	,, ,,
2	,,	43	,, ,,
74			

Results.—The average duration of menstrual (reproductive) life among Indian women does not appear to differ materially from the limits for European races.

Conclusions.

1. Among 489 Indian women representing many different castes and races, the average age of the onset of puberty (catamenia) was 13·63 years. The age of onset of puberty for Indian women lies within the limits for European races.

2. The average age at marriage among the same women was 13·83 years. Early marriage does not appear to exert any appreciable influence on the onset of puberty, since the average age of onset remains practically constant among Mussalmans (140 cases) 13·64 years, Hindus (268 cases) 13·62 years, and Indian Christians (81 cases) 13·69 years: although the age of women at marriage among the last group, 16·82 years, is higher than among either Mussalmans, 13·84 years, or Hindus, 12·91 years.

It is also noteworthy that the average age of onset among a group of unmarried women students (56 cases), 13·87 years, resembles the average age among the married women, 13·63 years.

3. The average duration of menstrual (reproductive) life among Indian women (74 cases), 32·14 years, does not appear to differ materially from the limits for European races.

The earlier age at marriage among Indian women does not appear to influence the duration of their reproductive or menstrual life as compared with European races.

My thanks are due to Dr. M. I. Balfour, W. M. S. I., and the Dufferin Fund Council for encouragement to make this enquiry, and to the many medical women who so kindly helped to obtain the necessary data.

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THE CORRELATION BETWEEN THE CHEMICAL COMPOSITION OF ANTHELMINTICS AND THEIR THERAPEUTIC VALUES IN CONNECTION WITH THE HOOKWORM INQUIRY IN THE MADRAS PRESIDENCY.

BY

Rev. Father J. F. CAIUS, S.J., M.S.C.I. (Paris),
Professor, St. Joseph's College, Trichinopoly,

AND

Dr. K. S. MHASKAR, M.D., M.A., B.Sc., D.P.H., D.T.M. & H.,
Officer in charge Ankylostomiasis Inquiry in the Madras Presidency.

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VII. OLEUM EUCALYPTI.

History.

It is mostly in combination with chloroform that oil of eucalyptus has been used as an anthelmintic in ankylostomiasis. The few cases in which it was administered alone were meant for the purpose of investigating its mode of action in the combined treatment.

Schultz (1911)[1] concluded from its action on dogs that eucalyptus is probably superfluous and at best is simply an aid to chloroform. Darling, Barber, and Hacker (1918)[2] stated that eucalyptus ranks very low as a vermicide, but, nevertheless, seems to aid chloroform in its action.

Composition and Properties.

Oil of eucalyptus is distilled from the fresh leaves of various species of *Eucalyptus*. The most important of these is *E. globulus* on account of its high eucalyptol content. Eucalyptol, which has to a considerable extent replaced the oil, is obtained by redistilling from potassium hydroxide or calcium chloride that portion of the oil which comes over between 170° and 178° C.

There is much variation in the physical constants found in the literature of the eucalyptus oils as determined by different observers. The physical data for the oils we have been using are recorded in Table I.

Table I.

Physical and Chemical Examinations of various samples of Oil of Eucalyptus.

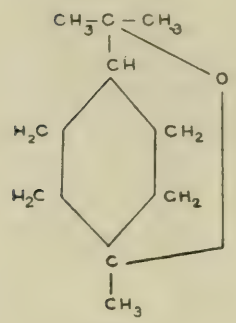
	Specific Gravity 29° C.	Optical Rotation 28° α_D	Refractive Index 28° μ_D	Solubility in alcohol 80 per cent	Eucalyptol content per cent	Phellandrene content per cent
Oil Eucalyptus II..	0·9093	+ 2·0°	1·4639	1 : 1·5	85	0
„ „ III..	0·9092	+ 2·3°	1·4608	1 : 2	78	0
„ „ IV..	0·9086	+ 8·7°	1·4589	1 : 1·5	82	0
Oil Eucalyptus globulus V ..	0·8976	+20·0°	1·4675	1 : 12	65	0
Oil Eucalyptus citriodora ..	0·8988	+18·8°	1·4720	1 : 1 90%	0	0

The oil from *E. globulus* contains *pinene*, *eucalyptol* or, as it is now generally known, *cineol*, and aldehydes of the fatty series. The cheaper oil of *E. amygdalina* is often used as a diluent, but its presence may be shown by the detection of *phellandrene*, one of its chief constituents. All our samples were free from phellandrene.

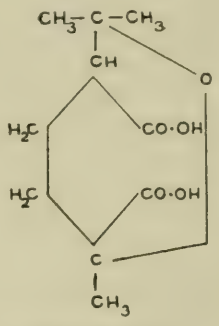
When taken in overdose, oil of eucalyptus causes rapidity of the pulse with general excitement and restlessness, nausea, vomiting, frequently dilatation of the pupils, and symptoms of collapse.

Because of the high repute eucalyptus has been held in, ever since the introduction of eucalyptus-chloroform mixtures against hookworms, we felt it incumbent on us to try different lots of oil. Four of them were official as *Oleum eucalypti*, and a fifth one, obtained from a reliable firm in India, was labelled *Eucalyptus globulus*. For the sake of comparison we have also used oil of *E. citriodora* which consisted mostly of *citronellal*, and contained no cineol. We regret our inability to

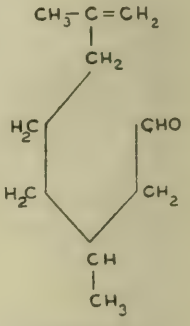
procure amygdalina oils, for nothing definite is known concerning the physiological action of phellandrene.



CINEOL



CINEOLIC ACID



CITRONELLAL

Absorption and Elimination.

By far the greatest amount of the ingested oil is eliminated through the breath to which it imparts its odour. The rest is passed with the urine as cineolic acid, an oxidation product of cineol.

There was no appreciable variation in the volume or the density of the urines after the eucalyptol treatment, and no characteristic odour or colour was noticed.

Anthelmintic Value.

Our experiments were carried out on adult and apparently healthy male prisoners, who were given the oil, together with one and a half ounce of castor oil, well-shaken with mucilage of gum acacia. The dose was divided into two portions which were administered at half an hour's interval. The alimentary canal had previously been prepared by purgation with Epsom salts. No food was allowed until the bowels had moved. As was naturally expected, the ready and efficacious action of the castor oil excluded recourse to an after-purge.

Starting with 30 minims, the dose prescribed in the weak formulæ of eucalyptus-chloroform mixtures—Hermann's, Manson's—we first ascertained that 60 minims caused uncomfortable giddiness, retching, and a feeble pulse. We, accordingly, adopted 40 minims as a safe dosage for the study of the anthelmintic value of the various samples of oil. This dosage, moreover, fairly agrees with that recommended in the strong formulæ of the mixture—Philips's, Schuffner's—.

The total hookworm content was determined in the usual routine way using 60 grains thymol as subsequent standard treatment.

TABLE II.

Number of Hookworms removed by one Test Treatment of Oleum Eucalypti.

Experiment number.	Test Treatment.		Number of cases treated.		Hookworms removed.			Percentage of Hookworms removed with a Test Treatment.		
	Oil Eucalyptus number.	Dose.			A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.
1	I	30 minims ..	11	Test treatment ..	0	6	6	0·0	0·8	0·7
				Subsequent treatments	108	734	842			
				Total hookworms ..	108	740	848			
2	II	40 minims ..	12	Test treatment ..	0	2	2	0·0	0·9	0·9
				Subsequent treatments	11	209	220			
				Total hookworms ..	11	211	222			
3	„	60 minims ..	10	Test treatment ..	0	9	9	0·0	1·3	1·3
				Subsequent treatments	20	643	663			
				Total hookworms ..	20	652	672			
4	III	40 minims ..	23	Test treatment ..	0	19	19	0·0	2·1	1·9
				Subsequent treatments	106	868	974			
				Total hookworms ..	106	887	993			
5	IV	40 minims ..	22½	Test treatment ..	0	11	11	0·0	1·8	1·8
				Subsequent treatments	71	581	652			
				Total hookworms ..	71	592	663			
6	V	40 minims ..	25	Test treatment ..	1	25	26	2·4	9·3	8·4
				Subsequent treatments	41	243	284			
				Total hookworms ..	42	268	310			
7	E. citri-odora.	40 minims ..	20	Test treatment ..	0	3	3	0·0	1·0	1·0
				Subsequent treatments	12	281	293			
				Total hookworms ..	12	284	296			

The test treatments, as recorded in Table II, show that oil of eucalyptus is ineffective for the removal of hookworms.

It is equally ineffective for the removal of other intestinal worms, since one case only passed ascarids though roundworms were present in 59 cases and whipworms in 30.

Darling, Barber, and Hacker had also concluded from their experiments with eucalyptus that it does not remove worms to any extent. With a 30 minims dosage the worms expelled by the trial treatment were 0 per cent in one group of seven patients, and 2·7 per cent in a second group of eight.

That oil of eucalyptus is a very weak vermicide may easily be gathered from the large number of worms which are expelled alive.

Mode of Action.

Notwithstanding the fairly large amount of literature on the subject, little is known with certainty of the medicinal and toxic effects of eucalyptus oil. However, in the belief that the medicinal properties depend upon cineol, many prefer to use the pure eucalyptol to the oil itself. Starting with 10 minims, the maximum dose prescribed, we have, accordingly, treated 125 cases with increasing dosages of eucalyptol in order to ascertain, as far as possible, whether or not the therapeutic virtues of the official oil are due to its cineol. The method of treatment was exactly the same as that we had previously adopted when working with the oil.

The first striking result was in the finding that pure eucalyptol is less toxic than the oil. We are inclined to believe that the aldehydes present in the oil are responsible for its higher toxicity.

It will be seen from Table III that cineol is the active principle of the oil: but that, in the therapeutic doses used in the eucalyptus-chloroform mixtures against hookworms, *viz.*, 30 and 10 minims, it has very little value as an anthelmintic. It is equally inefficient against other intestinal worms; for out of 52 cases infected with roundworms and 39 harbouring whipworms, only one passed ascarids. These, as also many of the hookworms, were expelled alive.

The eucalyptol treatment brought temporary relief to three cases of urethritis due to chronic gonorrhœa.

TABLE III.

Number of Hookworms removed by one Test Treatment of Eucalyptol.

Experiment number.	Test Treatment.	Number of cases treated.		Hookworms removed.			Percentage of Hookworms removed with a Test Treatment.		
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	A. americanus.	A. duodenale and N. Americanus.
1	10 minims ..	20	Test treatment ..	0	1	1	0·0	0·1	0·1
			Subsequent treatments	71	985	1056			
			Total hookworms ..	71	986	1057			
2	20 minims ..	16	Test treatment ..	0	1	1	0·0	0·3	0·2
			Subsequent treatments	23	324	347			
			Total hookworms ..	23	325	348			
3	30 minims ..	24	Test treatment ..	0	59	59	0·0	3·2	3·1
			Subsequent treatments	75	1750	1825			
			Total hookworms ..	75	1809	1884			
4	40 minims ..	25	Test treatment ..	1	69	70	2·1	8·1	7·7
			Subsequent treatments	46	774	820			
			Total hookworms ..	47	843	890			
5	50 minims ..	22	Test treatment ..	2	72	74	4·4	13·2	12·6
			Subsequent treatments	43	471	514			
			Total hookworms ..	45	543	588			
6	60 minims ..	9	Test treatment ..	0	62	62	0·0	36·9	32·3
			Subsequent treatments	24	106	130			
			Total hookworms ..	24	168	192			
7	90 minims ..	9	Test treatment ..	6	54	60	50·0	58·1	57·1
			Subsequent treatments	6	39	45			
			Total hookworms ..	12	93	105			

Conclusions.

1. Eucalyptus oil is a mixture of several constituents, not always the same, which may exist in widely varying proportions.

2. Most of the eucalyptus oils contain cineol to which they owe their medicinal properties. They also contain toxic compounds, probably aldehydes, which are not to be found in eucalyptol. Pure eucalyptol ought to be used preferably to the oil itself.

3. Eucalyptol possesses such very weak vermicidal properties that a strong purgative has to be administered along with it to ensure the removal of the worms before they have time to recover from the effects of the drug.

4. Eucalyptol ranks low as an anthelmintic and cannot be recommended for use in the eradication of the hookworm infection.

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Officer in charge Ankylostomiasis Inquiry in the Madras Presidency.

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VIII. CHLOROFORM.

History.

In 1897, Carratu[1] drew attention to the value of the internal administration of chloroform in cases of tapeworm, and adopted a mode of treatment first described by an American writer in 1888. Moderate diet for two days—but no fasting; and then, in the course of eight hours, the patient takes from 45 minims to one drachm of pure chloroform in syrup, a quarter of it at each dose, and a dose every two hours; after that a dose of castor oil is given. There were no symptoms of poisoning and no ill effects.

Schultz (1911)[2] found that chloroform was very effective against hookworms in the dog, and stated that should the chloroform-castor

oil mixture act as favourably in human beings, it would prove a universal worm remedy of great importance. However, Schultz considered chloroform a dangerous drug when taken in doses usually recommended for expelling intestinal worms and, afraid as he was of the immediate injuries following overdoses of this drug, he favoured the use of thymol.

Hall and Foster (1917)[3] say that Schultz's experimental results favouring chloroform made little impression in America, and that the enthusiastic advocacy of chloroform as an anthelmintic came from an Italian, Alessandrini. Alessandrini[4] advocates the use of from 3 to 4 grammes doses, dissolved in olive or castor oil, and states that it is thoroughly efficatious against hookworms, whipworms, pinworms, and ascarids. The advantages of chloroform are the following:— combined with castor oil, it requires no special preparation of the patient; it can be administered at a single dose; it does not cause local or general disturbances, either immediately or subsequently; and it is perfectly well tolerated and not nauseous.

Hall and Foster confirm the efficacy of chloroform against hookworms and thus conclude their paper: 'Chloroform in castor oil, in therapeutic doses, is the most effective anthelmintic we have found for use against hookworms, and we consider it as safe as thymol or any other effective drug for use against hookworm disease.'

Darling, Barber and Hacker (1918)[5] treated a group of eight patients with 15 minims pure chloroform in order to investigate its mode of action in the eucalyptus-chloroform mixtures, and they concluded that the greater part of the effect of the combined treatment is due to the chloroform, which by itself brought away 30 per cent of worms. In another series of eight, chloroform, following upon a first trial treatment of eucalyptus, removed 49·3 per cent of the worms.

Anthelmintic value.

Various samples of chloroform were carefully tested for impurities, and only pure chloroform was used in this investigation.

Apparently healthy adult male prisoners were treated with varying dosages of pure chloroform well-shaken with 12 drachms of castor oil in mucilage acacia. The patient was given a saline purge the night previous to the treatment, and in the early morning took the drug in two portions, at half an hour's interval. No after-purge followed, and food was not allowed before the bowels had moved.

The total hookworm content was determined in our usual routine way using 60 grains thymol as subsequent standard treatment.

TABLE I.

Number of Hookworms removed by one Test Treatment of Chloroform.

Experiment number.	Test Treatment.	Number of cases treated.		Hookworms removed. A. duodenale.	Hookworms removed. N. americanus.	Hookworms removed. A. duodenale and N. americanus.	Percentage of Hookworms removed with a Test Treatment. A. duodenale.	Percentage of Hookworms removed with a Test Treatment. N. americanus.	Percentage of Hookworms removed with a Test Treatment. A. duodenale and N. americanus.	Percentage of cases cured of hookworm infection with a test treatment.
1	15 minims..	22	Test treatment..	2	14	16	8·0	3·4	3·6	0·0
			Subsequent treatments ..	23	404	427				
			Total hookworms	25	418	443				
2	30 minims..	20	Test treatment..	15	65	80	53·5	14·2	16·5	15·0
			Subsequent treatments ..	13	393	406				
			Total hookworms	28	458	486				
3	40 minims..	24	Test treatment..	16	171	187	76·2	48·9	50·3	16·6
			Subsequent treatments ..	5	179	184				
			Total hookworms	21	350	371				
4	45 minims..	12	Test treatment..	72	684	756	92·2	86·0	86·7	25·0
			Subsequent treatments ..	5	111	116				
			Total hookworms	77	795	872				

It will be seen from Table I that chloroform is decidedly effective as an anthelmintic against hookworms, and is particularly potent against *Ankylostoma*.

Within the limits of the 30—45 minims doses, we found chloroform little effective against roundworms—only two cases passed worms out

of 40 infected—, and not effective against whipworms—no worms were expelled though 26 cases were found to be infected.

The rationale of the chloroform treatment.

1. *Toxicity to the worms.*—Most of the worms are expelled alive, and the few dead ones are usually flaccid, very rarely contorted. Chloroform is evidently not a vermicide. Nor is it a vermifuge. Chloroform is essentially an anæsthetic and acts as such on the parasites, benumbing and, exceptionally, killing them. A vigorous purge is therefore necessary in order to sweep the worms out before they have time to recover from the stupefying effect of the drug.

As narcosis is not selective in its action, it follows that *Ankylostomum* is not more chloroform resistant than *Necator* ; and this naturally places chloroform very high as an anthelmintic whenever *A. duodenale* is mostly responsible for the infection.

2. *Toxicity towards the host.*—Chloroform is not only poisonous in a state of vapour, but also when taken into the stomach as a liquid. Our experience is that, even with the smaller 45 minims dose advised—Hermann's Mixture, Manson's Mixture—choroform is an unpleasant drug to handle. Toxic symptoms are occasionally noticed with a dose of 30 minims and become more general and more marked with increasing dosages until 45 minims is reached. The after effects of chloroform medication were *nil* in the cases we treated.

Chloroform treatment is, therefore, such as to necessitate hospital conditions.

We are of opinion that, with subjects hardier and more addicted to alcohol than are the Tamilians of Southern India, larger doses of chloroform might safely be used. It has been our experience throughout the course of this investigation that prisoners addicted to alcohol, when at large, suffer less from the immediate effects of chloroform medication, and that their immunity was relatively proportional to the frequency of their potations and the quantities they would imbibe. Of course, there is nothing in this to surprise us, when we bear in mind that both alcohol and chloroform are narcotics, and that in the cases of slow poisoning the symptoms are the same with the two drugs, the only difference being in the duration of the stimulation period which is excessively short with chloroform. That habituation to the effects produced on the nervous system by alcohol should lead to a better tolerance of chloroform seems natural.

Conclusions.

1. Pure chloroform in *combination with castor oil* ranks high as an anthelmintic against hookworms.

2. Provided hospital conditions are possible the chloroform treatment recommends itself wherever *ankylostome* infection prevails.

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IX. THE EUCALYPTUS-CHLOROFORM MIXTURE.

History.

Hermann was the first to substitute eucalyptus for male fern in the treatment of ankylostomiasis in the mines of Mons, in Belgium. The treatment lasted eight days. On the first day the patient was given on an empty stomach

Eucalyptus	..	..	..	..	2 grammes.
Chloroform	..	..	..	..	3 grammes.
Castor oil	..	..	..	..	40 grammes.

Two days after, 100 grammes chloroform water (1 per cent) and 10 grammes sodium sulphate were administered in four portions at

intervals of fifteen minutes. This was repeated two days after. And three days later, another dose of the eucalyptus-chloroform mixture was given together with castor oil[7].

In 1905 Phillips[2] introduced into Egypt his eucalyptus mixture which, for a time, entirely superseded thymol. He modified Hermann's Mixture by increasing the eucalyptus to 2·5 and the chloroform to 3·5 grammes. In the evening, he gave a saline purge—which he has often omitted—and kept the patient fasting all night. Half a dose of the mixture was administered at 7 A.M. and the second half at 7-30 ; and the patient was kept in bed, still fasting, until stools were passed. Occasionally the first administration was not successful and Phillips could not account for the failure. As the worms were expelled alive, he suggested that in a few cases the worms may have time to recover and refix themselves. That the chloroform is essential was shown by the fact that in three cases chloroform water was given instead of chloroform and no worms were passed.

Shattuck used Phillips' Mixture in the Bilibid Prison, Manila, during the years 1906–1908, and Castellani (1906)[1] in Ceylon, wrote :—'I can confirm that the chloroform-eucalyptus oil mixture gives very good results in the treatment of this disease (ankylostomiasis).'

However, Castellani's favourable opinion is not shared by Ashford and Igarravidez[3] of the Porto Rico Anæmia Commission (1907), who say :—'Our experience with eucalyptus oil in a few cases does not permit us to advise the use of this drug, so highly lauded by Phillips :

Eucalyptus oil	..	..	2·50 grammes.
Chloroform	..	..	3·50 grammes.
Castor oil	..	..	40·00 grammes.

The medicine was administered every two days, the dose being divided into three equal parts and one dose being taken every 20 minutes, the intestines of the patient having been previously emptied by means of a purgative. All the patients suffered from dizziness, fatigue, and a desire to sleep ; and in some vomiting, in others syncope, was produced, making it necessary to administer stimulants to prevent a fatal result. Without inferring that its anthelmintic power is inferior to that of thymol and betanaphthol, our experiments enable us to state that it is dangerous, not easily administered, costly, and unfit to dispense in the country where the peasant has to carry his medicine a long distance from the station. Nevertheless, the use of eucalyptol affords the great advantage of enabling us to secure living parasites.'

Drummond (1908)[4] mentions a method of giving eucalyptus oil and castor oil adopted in Ceylon. Starve the patient the day previous and give an aperient of castor oil. Then, in the early morning, mix 30 drops of eucalyptus oil and spirits chloroform 30 drops into 12 drachms of castor oil, and give in three-hourly portions. Repeat on third and seventh, fourteenth and twenty-first day. For a child, 5 drops eucalyptus and 6 drachms of castor oil are required.

Hermann's Mixture was soon introduced into India where it became known as Manson's Mixture, and, as early as 1909, Clayton Lane[6] mentioned the vermifugal action of the drug in a case where three doses of thymol of 30 grains each at intervals of an hour had failed to expel any ankylostoma. Manson himself declared the eucalyptus-chloroform mixture to be a very efficient vermifuge, much less unpleasant and much less dangerous than thymol or betanaphthol, and which could be repeated for several days in succession.

Jolly (1910)[8] was, however, unfortunate in his use of the mixture, and he records a case of poisoning by it. The patient was treated according to Manson's prescription. After the two doses at half hour's interval, the patient kept well till 9-30 A.M., when he stated he felt weak and giddy. At 10 A.M. he collapsed; languor, giddiness, difficulty of breathing, and pain over the body; breath smelling strongly of eucalyptus; no vomiting; neither urine nor feces passed. The patient recovered after stimulant treatment. Jolly hesitated to adopt the eucalyptol reatment in another case.

Thomas (1910)[9] discouraged the use of Mons' Mixture * for children, as the drowsiness induced by chloroform-eucalyptus is apt to alarm the parents.

Schultz (1911)[10] reports that acute mania resulted in a boy who took an adult dose of Hermann's Mixture, and he says that Colbert, Key and Law speak of it as dangerous and less efficient than betanaphthol. He thinks that eucalyptus acts as an irritant on the parasites causing them to loosen their hold, chloroform anæsthetizes them, and a vigorous purge sweeps them out. He concludes that eucalyptus is probably superfluous and at best is simply an aid to chloroform.

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* In 1908 Sandwith[5] had stated that 'since the introduction of thymol into Egypt a new drug had sprung up which, he believed, Mons, a Belgian, was the first to introduce, namely, eucalyptus with castor oil and chloroform, which certainly was a much safer remedy.'

Burton Nicol (1912),[11] after a careful study of the effect of various drugs in expelling hookworms, states that eucalyptus proved very unsatisfactory. Some cases, after the first treatment, commenced retching, had nausea and vomiting, dizziness, syncope and somnolence.

Tar pore[12] does not find eucalyptus oil and chloroform successful and records intoxication and collapse in one case.

Schüffner and Vervoort[16] used :—

Eucalyptus	3 grammes.
Chloroform	3·50 grammes.
Castor oil	40 grammes.

in two portions at half an hour's interval and found that 38 per cent of the worms were expelled.

Aubrey[17] gave :—

Eucalyptus	2 grammes.
Chloroform	4 grammes.
Castor oil	40 grammes.

in three doses at two hours' interval. A cure was effected in 52·25 per cent of the cases after one treatment.

Summarising the reports of surgeons in East Africa, who had each treated some hundreds of cases by four different drugs—betanaphthol, thymol, eucalyptus oil, and male fern—Greisert[13] concludes that eucalyptus oil fails to commend itself.

The treatment first adopted by Johnston[14] in Northern Nigeria was 6 drachms of the eucalyptus, castor oil and chloroform mixture in the early morning, repeated after an hour and a half, the double dose being again given three days later. This se med quite inefficient, and better results attended the administration of thymol in divided doses.

Saundby[15] gave Hermann's Mixture to his Birmingham case. It was divided into two doses taken at an interval of one hour, and preceded and followed by sodium sulphate. The patient complained of the mixture as very nauseous, and had comated slightly after the second dose.

In Guiana, Blin[18] gave the eucalyptus-chloroform mixture to debilitated patients ; but Theze[21] completely abandoned it on account of its inconsistency and the difficulty of its administration.

In the course of the year 1915, Eldred[19] reports 67 per cent cures obtained with the eucalyptol treatment, Clayton Lane[20] speaks very favourably of it, and Moillet and Carrero[22] find that it gives good results in all but very obstinate cases. Lane says that the

treatment paralyses rather than kills the ankylostomes, and the castor oil is an essential part of it; for the ankylostomes must be expelled before they recover from the stupefying effect of the drugs and attach themselves again to the wall of the gut.

In 1918, Darling, Barber, and Hacke,[23] published the results of an investigation of the mode of action of eucalyptus and chloroform and thus summarised the results: 1, Eucalyptus ranks very low as a vermicide; 2, the greater part of the effect of the combined treatment is due to the chloroform; 3, on the other hand, although eucalyptus does not remove worms to any extent, it seems to aid chloroform in its action.

Wrench (1918)[25] gave Manson's Mixture on three successive mornings in the manner prescribed by Manson to nineteen cases. Six treatments failed to 'cure' a single case, and Wrench concludes that, for the purpose of sterilization, Manson's Mixture is useless.

Surveyor (1919)[24] says it was noticed that the original Egyptian Mixture has practically not much effect in eradicating the parasites. So it was decided to increase the strength of the mixture, and the modified form adopted is the following:—

Ol. Eucalypti	75 minims.
Chloroform Pur.	90 minims.
Ol. Ricini	20 drachms.
Tinct. Digitalis	20 minims.

This is divided into four parts. The first dose is given in the early morning and the remaining three doses repeated each at an interval of one hour. The pulse and general condition of the patient are watched carefully before giving each dose. In the second treatment, two doses are given early in the morning and the remaining two doses are repeated, each at an interval of one hour, with the same precautions as mentioned above. And this is repeated twice a week till no ova or parasites can be detected. According to Surveyor, this method, on the whole, appears to be fairly satisfactory and free from danger, and it was successfully administered to a case suffering from acute Bright's Disease and ascites due to cirrhosis of liver.

Anthelmintic Value.

Healthy adult male prisoners were treated with Hermann's Mixture emulsified with gum acacia. The dose was administered in two

portions with an interval of half an hour. The treatment was preceded by an overnight purge of Epsom salts. The after-purge was found unnecessary as the castor oil in the mixture acted efficiently and rapidly. The patient was kept fasting until stools were passed.

The total hookworm content was determined in the usual way, using 60 grains thymol as subsequent standard treatment.

Our results compare very favourably with those previously obtained by various authors.

TABLE I.

Number of Hookworms removed by one Test Treatment of the Eucalyptus-Chloroform Mixture.

Experiment number.	Test Treatment.	Number of cases treated.		Hookworms removed.			Percentage of hookworms removed with a test treatment.			Percentage of cases cured of hookworm infection with a test treatment.
				A. duodenale.	N. americanus.	A. duodenale and N. americanus.	A. duodenale.	N. americanus.	A. duodenale and N. americanus.	
1	Ol. Eucalypti minims 30 .. Chloroform minims 45 .. Ol. Ricini 3 12 .. Mucilage—q.s. in two portions at half-an hour's interval.	36	Test treatment..	100	1822	1922	77·5	78·6	78·5	16·6
			Subsequent treatments.	29	496	525				
			Total hookworms	129	2318	2447				

THE RATIONALE OF THE EUCALYPTUS-CHLOROFORM TREATMENT.

1. *Worm removal and "cures."*—In our hands Hermann's Mixture expelled 78·5 per cent of the hookworms. This, we believe, is the highest figure ever recorded. Nevertheless, it falls short of the 86·7 per cent hookworm removal we obtained with 45 minims of chloroform alone without eucalyptus.

TABLE II.

Percentage of Hookworms removed by one Test Treatment of the Eucalyptus-Chloroform Mixture.

No.	Authors.	People experimented on.				Hookworm Infection.		Ol. Eucalypti 2·0 gms. Chloroform 3·0 gms. Ol. Ricini 40·0 gms. in two portions at half hour interval.	Ol. Eucalypti 2·5 gms. Chloroform 3·5 gms. Ol. Ricini 40·0 gms. in two portions at half hour interval.	Ol. Eucalypti 3·0 gms. Chloroform 3·5 gms. Ol. Ricini 40·0 gms. in two portions at half hour interval.
		Nationality.	Sex.	Age.	Health.	Infection.	Species formula.			
1	Burton Nicol (1912) ..	Indians	Males	Adults	Coolies	26 hookworms per case	?	....	74·2 10 cases.	
2	Schüffner and Vervoort (1913).	?	?	?	?	?	?	....	....	38·6 354 cases.
3	Darling, Barber and Hacker (1918)	Chinese, Malayese	Males	Adults	Healthy prisoners	58 hookworms per case	88 Necators to 12 Ankylostomes.	46·9 10 cases.		
4	Caius and Mhaskar (1917-1920)	Tamil Indians	Males	Adults	Healthy prisoners	55 hookworms per case	94 Necators to 6 Ankylostomes.	78·5 36 cases.		

We have already noted in previous papers that, while eucalyptus has no action on ankylostomes and very little on necators, chloroform is equally efficient against both kinds of worms. Table I shows a 77·5 per cent ankylostome removal as compared with a 78·6 per cent necator removal.

If we now turn to the number of 'cures' we obtained with Hermann's Mixture, we find a percentage of 16·6 as against 25·0 recorded with 45 minims of chloroform alone. Aubrey obtained 52·3 per cent cures with a mixture which contained 60 minims chloroform.

TABLE III.

Percentage of Cures obtained with one Test Treatment of the Eucalyptus-Chloroform Mixture.

No.	Authors.	People experimented on.				Hookworm Infection.			Ol. Eucalypti 2·0 gms. Chloroform 3·0 gms. Ol. Ricini 40·0 gms. in two portions at half hour interval.	Ol. Eucalypti 2·0 gms. Chloroform 4·0 gms. Ol. Ricini 40·0 gms. in three portions every two hours.
		Nationality.	Sex.	Age.	Health.	Infection	Species formula.	Method of diagnosing hookworm ova infection.		
1	Aubrey, G. E. (1913)..	Hongkong	..	..	..	..	....	....	....	52·3 ? cases.
2	Wrench G. T. (1918)..	Indians	Males	Adults	Sepoys as patients in the hospital	?	?	Telman's process examined up to six weeks after treatment.	0·0 19 cases.	
3	Caius and Mhaskar (1917–1920)	Tamil Indians	Males	Adults	Healthy prisoners.	55 hookworms per case.	94 Necators to 6 Ankylostomes.	Howard's process examined twelve or more days after treatment.	16·6 36 cases.	

It is evident, then, that in the eucalyptus-chloroform mixture, chloroform alone has an anthelmintic value and that eucalyptus is superfluous and may be dispensed with altogether.

Schüffner and Vervoort noted that 8 out of 162—12 per cent—ascarids harboured by their 347 cases were expelled by the eucalyptus-chloroform mixture. Out of nine of our cases in which roundworms were pre ent, one case passed one worm on the third day after the treatment. The mixture cannot be recommended as an anthelmintic against worms other than hookworms.

2. *Toxicity towards the worms.*—Many of the worms are expelled alive, thus showing that the mixture is a very weak vermicide. The castor oil is, therefore, an essential part of the mixture.

3. *Toxicity towards the host.*—We found the mixture highly toxic to the host : in view of this, we did not feel justified in trying any of the larger doses mentioned in the literature.

Conclusions.

1. The eucalyptus-chloroform mixture, in the doses usually prescribed, is a mixture in which one of the constituents, eucalyptus, is superfluous.

2. The suppression of eucalyptus, without in any way lowering the anthelmintic value of the treatment, would lessen its toxicity, reduce its cost, and facilitate its administration.

3. The mixture has a comparatively low anthelmintic value and a comparatively high toxicity.

4. The toxic and anthelmintic properties reside in the same constituent, chloroform, and are therefore inter-dependent.

5. As the active constituent does not kill the worms but simply anæsthetizes them, a strong purge is necessary to expel the parasites.

6. Under the conditions, there will always subsist an element of doubt as to both the safety and the efficiency of the treatment.

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THE PREVALENCE OF ANKYLOSTOMIASIS IN THE MADRAS PRESIDENCY.

BY

DR. K. S. MHASKAR, M.D., M.A., B.SC., D.P.H., D.T.M. & H.,

Officer in charge Ankylostomiasis Inquiry in Madras.

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III. HOOKWORM INFECTION AS A PREDISPOSING FACTOR IN MEDICAL DISEASES.

THE investigations in the Trichinopoly Jail, mentioned in my previous paper[1] show that freedom from hookworm infection seems to diminish the susceptibility to bowel complaints. The investigations carried out to the end of the year 1919 support the above conclusion; they also show that the average death rate and the average period of illness from bowel complaints are likewise lessened.

In Table I are recorded the total cases, total deaths and the average period of illness per case for the years 1918 and 1919 in the two groups of convicts—those free from, and those infected with, hookworm. The mille rates for case incidence and deaths are based on the respective average population of the two groups during the period. Except for the matter of treatment or nontreatment the two groups 'treated' and 'untreated' are otherwise exactly comparable.

TABLE I.

Case rate and Death rate from Bowel complaints in the Treated and Untreated for Hookworm.

	Treated for Hookworm.			Untreated for Hookworm.		
		Rate per mille.			Rate per mille.	
1918.						
Average population..	553	..	..	550	..	..
Total cases ..	..	64	113·5	..	112	203·6
Total deaths ..	..	5	8·0	..	10	18·1
Average period of illness in *days* ..	..	15·6	..	..	14·1	..
1919.						
Average population..	654	..	..	724	..	..
Total cases ..	..	69	105·9	..	140	193·3
Total deaths ..	..	0	0·0	..	8	11·0
Average period of illness in *days* ..	..	10·7	..	..	15·1	..

An epidemic of influenza in the jail gave an opportunity to observe whether the susceptibility to it is influenced by the presence or absence of hookworm infection in the host. The epidemic lasted for six months from July to December 1919, was at its height in November, and was characterized throughout by the frequency and severity of lung complications. A total of 370 cases and 32 deaths that occurred during the period are classified in Table II under the same headings as shown for bowel complaints.

TABLE II.

Case rate and Death rate from Influenza in the Treated and Untreated for Hookworm.

	Treated for Hookworm.			Untreated for Hookworm.		
		Rate per mille.			Rate per mille.	
July 1919–December 1919						
Average population .	665	..	..	706	..	..
Total cases ..	..	155	248·1	..	215	304·5
Total deaths ..	..	14	21·0	..	18	25·5
Average period of illness in *days* .	..	14·8	..	..	15·5	..

The figures show that freedom from hookworm infection seems to diminish the susceptibility to influenza infection ; they also support the conclusion arrived at by the Ceylon Commission,[2] who found the death rate from influenza to be 7·5 per thousand in the 14,659 persons who had been treated for hookworm and 13·8 per thousand among 3,253 persons who had not been treated for hookworm.

Conclusion.

Hookworm infection seems to be a predisposing factor in bowe complaints and in such a generalized infection as influenza.

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HOOKWORM INFECTION AND SANITATION.

BY

DR. K. S. MHASKAR, M.D., M.A., B.SC., D.P.H., D.T.M. & H.,

Officer in charge Ankylostomiasis Inquiry in Madras.

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IT has been pointed out in my previous papers [10, 13] that the average hookworm infection in a group of persons is proportional to the chances of infection arising from the surrounding local sanitary conditions as well as the individual habits and occupations. Thus the infection was found to be 91 per cent in the municipal town of Negapatam, and 98·7 per cent in the villages around. The percentage of infection was less in females than in males, less also in males who have in-door occupation than in those who lead an out-door life. In Dindigul town [13] the number of hookworms harboured per person was 11 in the upper and middle classes and 127 among the sweepers.

This paper shows that mere improvement in sanitary conditions without the help of any specific treatment is sufficient to bring down the intensity of hookworm infection.

We know from the life-history of the hookworm that the infection is concurrent with the life of the adult worm in the intestine : and so, were an infected person to remove himself to a place where re-infection is not possible, the parasite he brings with him would either die or be evacuated in course of time, and natural cure would follow.

Such natural cures are not altogether unknown. Ashford and Igarravidez[6] (1911) cite the case of the Porto Rico Jibaro, who, leaving their agricultural pursuits, came to a town and gradually found

themselves cured of their anæmia and improved in health. Ashford and King[3] (1907) also attribute to this natural cure the improvement in health of the soldiers of the Porto Rico regiment from the day they were removed from their infected homes and were compelled to wear shoes. Boycott[5] (1911) says that it has been frequently found—and in Cornwall the evidence is clear—that if an anæmic person is removed from the infected place, he often rapidly improves, and may recover completely. In the United States Government Hospital for the Insane, Garrison and others[2] (1903) found that the frequency of infection with all intestinal parasites diminished with the length of residence.

I have already described, in a previous paper,[13] the sanitary conditions of the Trichinopoly Jail, and shown that they could scarcely be improved upon. Taking advantage of the favourable circumstances in which I was thus placed, I have tried to determine whether sanitation has any bearing on hookworm infection. For the purpose of this investigation, I have distributed my work into two parts:—(1) the determination of the percentage of prisoners found infected on their admission into the jail and after increasing periods of stay there; (2) the determination of the number of hookworms harboured by new arrivals, and by residents with varying duration of jail life. 1878 Trichinopoly prisoners were examined. To these may be added 463 prisoners of the Coimbatore Jail, where the sanitary conditions are very much the same as in Trichinopoly.

By way of illustration, I have appended figures which throw light on the effect of sanitation upon the natural removal of nematodes other than hookworms.

I. Percentage of Hookworm Infected cases at different periods of stay in jail.

That the percentage of infected cases can be brought down by the improved sanitation was noted by Fearnside[1] (1900), who found ankylostome infection to be 72 per cent in 100 fresh admissions into the Rajahmundry Jail, and only 58 per cent in 200 convicts with a stay of six months or more. His method of examination consisted in the careful inspection of three slides. He considered re-infection in jail unlikely.

Tarapore,[7] working in the Mandalay Jail, found a 61 per cent infection in 1037 convicts admitted in 1910. In 1911 he examined 832 of the above convicts, and recorded an infection of 40 per cent. His

results were based on the examination of a loopful of feces under a coverslip.

Garrison[8] met with a 52 per cent infection in 4106 Bilibid prisoners. Re-examination of 930 cases after various lengths of stay in jail (less than one year, 241 cases; 1 to 2 years, 150 cases; 2 to 3 years, 356 cases) showed that only 5·9 per cent were still infected. He attributed this result to the sanitary condition of the institution.

Chamberlain[4] (1909) found the infection to be 85 per cent in new recruits to the army, and 11 per cent in 33 cases after three years' service.

Willets[9] (1917) reported that in Georgia Sanatorium the condition for the propagation of ankylostomes had been unfavourable. The percentage of infection diminished with length of stay under sanitary conditions as follows :—

Period of stay.	Cases examined.	Percentage of cases infected with hookworm.
30 days or less	500	22·2
31 days to 1 year	251	20·3
2 to 3 years	146	21·3
4 to 6 ,, ...	121	19·0
7 to 10 ,, ...	79	6·3
11 to 15 ,, ...	57	8·8
15 years and over	70	2·8

Acton[12] (1919) noted that the incidence of the ankylostomes amongst the personnel of the Indian General Hospital (Mesopotamia), which was 26 per cent in 1916 for 112 cases, had in 1918 dropped to 5·6 per cent for 300 cases. The reduction, he says, was really greater, as many of the infections found in 1918 were amongst those who were re-inforcements, and only had a few months' service in the country. He concludes that the longer the service in Mesopotamia the greater is the tendency for a natural cure. The technique was based on the microscopical examination of two loopfuls of fecal emulsion under a coverslip.

That the coverslip method fails to detect light infections is admitted by all. It may, therefore, be that with length of stay under sanitary

conditions the infection had been so reduced as to escape notice in the coverslip method of examination, and uncured cases were reported cured. This would explain why my results do not agree with those arrived at by the authors mentioned above.

TABLE I.

Incidence of Hookworm Infection at different periods of stay in the Trichinopoly and Coimbatore Jails.

Period of stay.	A. TRICHINOPOLY JAIL.			B. COIMBATORE JAIL (YEAR 1917).		
	Total cases examined.	Cases showing hookworm ova.	Percentage showing hookworm ova.	Total cases examined.	Cases showing hookworm ova.	Percentage showing hookworm ova.
0– 5 days ..	318	306	96·1	..	..	..
6–30 ,, ..	803	784	97·2	34	32	94·1
1– 2 months ..	187	178	95·1	22	21	95·4
2– 3 ,, ..	96	93	96·2	19	18	94·7
3– 6 ,, ..	105	95	90·4	54	49	90·7
6– 9 ,, ..	78	74	94·8	63	56	88·8
9–12 ,, ..	62	61	98·3	41	35	85·3
12–15 ,, ..	37	33	89·1	34	32	94·7
15–18 ,, ..	43	40	93·0	26	24	92·3
18–21 ,, ..	26	24	92·3	8	7	87·5
21–24 ,, ..	28	25	89·2	12	8	66·6
24–30 ,, ..	19	18	94·7	42	37	88·1
30–36 ,, ..	24	21	87·5	33	26	78·7
3– 4 years ..	16	15	93·7	43	34	79·1
4– 5 ,, ..	14	13	92·3	15	12	80·0
5– 6 ,, ..	5	5		3	2	
6– 7 ,, ..	4	3		7	7	
7– 8 ,, ..	4	3		4	3	
8– 9 ,, ..	3	3		1	1	
9–10 ,, ..	..	..		1	0	
10–11 ,, ..	1	0		..	..	
11–12 ,, ..	1	1	84·2	..	..	82·3
12–13 ,, ..	..	..		..	..	
13–14 ,, ..	..	..		..	..	
14–15 ,, ..	..	..		1	1	
15–16 ,, ..	..	..		..	..	
16–17 ,, ..	1	1		..	..	
TOTALS ..	1878	1796	..	463	405	..

My results, as shown in Table I, were obtained by a careful microscopic inspection of the stools, according to the centrifuge method—about a gramme of feces being minutely examined in all negative cases. They show that the incidence of hookworm infection is not appreciably affected by jail life under most suitable sanitary conditions.

Three main reasons may account for this :—

(*a*) the span of life of the worm extends beyond 16 years ;

(*b*) improved sanitary conditions do not exclude all possibility of re-infection ; and

(*c*) even on the supposition of no re-infection and on the contrary supposition of progressive elimination, we may still find the same percentage of cases affected by the few remaining worms : or, in other words, for the same duration of life under the same conditions of sanitation, practically all the infected convicts have passed from a higher to a lower degree of infection.

I find it impossible to give an opinion either about the duration of the life of the worm in the intestine or about the possibilities of re-infection under improved sanitary conditions ; but the next section bears out the fact that natural progressive elimination of the worms does take place and seems to be conditioned by the length of stay in jail away from the ordinary sources of infection.

II. Intensity of Hookworm Infection at different periods of jail life.

I have recorded, in the previous section, attempts by different authors at determining the bearing of jail life on the incidence of hookworm infection at different periods of stay in jail, and I have remarked that owing to the defective method of examination, light infections were possibly overlooked and uncured cases were probably reported as cured. The results arrived at do, nevertheless, point to a marked fall in the intensity of the infection with length of stay.

Darling,[11] working in the Batavia jail, found the average number of hookworms to be 110 in convicts with less than one year stay, 80 in those with a stay of 1 to 5 years, and 45 in those with a stay of 5 years and more. He came to the conclusion that there is a tendency for the number of hookworms to diminish as the length of stay under sanitary conditions increases.

My results agree with Darling's. They show that an infection, averaging 58 hookworms per case on admission, had fallen to 48 worms

per case after one month's stay in jail, and gradually dropped down to 32, 29, 17 worms per case after respective stays of 3, 12, 18 months. Beyond 18 months of jail life the number of hookworms per case remains fairly constant, and even such a long stay as 17 years does not seem to bring perfect sterilization of the host.

TABLE II.

Average number of Hookworms per case at different periods of stay in the Trichinopoly Jail.

Period of stay.	Total cases treated.	A. DUODENALE WORMS.		NECATOR WORMS.		A. DUODENALE AND NECATOR WORMS.	
		Total worms removed.	Average worms per case.	Total worms removed.	Average worms per case.	Total worms removed.	Average worms per case.
0– 5 days ..	318	1051	3·3	17332	54·5	18383	57·8
6–30 ,, ..	806	2580	3·2	35797	44·4	38377	47·6
1– 2 months ..	187	431	2·3	6211	33·2	6642	35·5
2– 3 ,, ..	96	202	2·1	2938	30·6	3140	32·7
3– 6 ,, ..	105	171	1·6	2795	26·6	2966	28·2
6– 9 ,, ..	78	110	1·4	2226	28·5	2336	29·9
9–12 ,, ..	62	118	1·9	1679	27·0	1797	28·9
12–15 ,, ..	37	64	1·7	632	17·1	696	18·8
15–18 ,, ..	43	88	2·0	662	15·2	750	17·4
18–21 ,, ..	26	38	1·4	278	9·6	316	11·0
21–24 ,, ..	28	86	3·1	293	10·3	379	13·4
24–30 ,, ..	19	28	1·4	251	13·2	279	14·6
30–36 ,, ..	24	26	1·0	348	14·5	374	15·5
3– 4 years ..	16	9	0·5	178	11·1	187	11·7
4– 5 ,, ..	14	6	0·4	153	10·9	159	11·3
5– 6 ,, ..	5	12	..	70	..	82	
6– 7 ,, ..	4	1		28		29	
7– 8 ,, ..	4	0		20		20	
8– 9 ,, ..	3	2		2		4	
9–10 ,, ..	..	..	0·8	..	6·4	..	7·3
10–11 ,, ..	1	0		0		0	
11–12 ,, ..	1	0		3		3	
12–16 ,, ..	..	..		..		..	
16–17 ,, ..	1	1		0		1	
	1878	..	..	..	..	..	..

All I can say with certainty is that prolonged life under improved sanitary conditions naturally brings down the intensity of hookworm infection.

III. '*Ascaris*' and '*Trichiuris*' Infections at different periods of jail life.

Fearnside[1] (1900) noted a 36 per cent *Ascaris* infection brought down to 18·5 per cent by a stay of six months in the Rajahmundry Jail. Garrison[8] found a 26 per cent *Ascaris* infection, and a 59 per cent *Trichiuris* infection in 4106 new convicts; on re-examination, a year or more after incarceration, the infections were 6·5 per cent and 26·7 per cent, respectively.

Willets[9] (1917) reported that whilst the conditions in the Georgia Sanatorium were unfavourable for the propagation of ankylostomes, they appeared to be favourable for *Trichiuris* and *Ascaris*.

Periods of stay.	Cases examined.	Percentage of cases infected with *Trichiuris*.	Percentage of cases infected with *Ascaris*.
30 days or less ..	500	0·6	2·4
31 days to 1 year ..	251	16·3	21·9
2 to 3 years ..	146	43·2	28·1
4 to 6 ,, ..	121	56·2	29·8
7 to 10 ,, ..	79	55·7	36·2
11 to 15 ,, ..	57	59·7	21·1
15 years or more ..	70	57·2	27·2

TABLE III.

Percentage of Ascaris *and* Trichiuris *infections at different periods of stay in the Trichinopoly and Coimbatore Jails.*

Period of stay.	A. Trichinopoly Jail.					B. Coimbatore Jail (Year 1917).				
	Total cases examined.	Cases showing *Ascaris* ova.	Percentage of cases showing *Ascaris* ova.	Cases showing *Trichiuris* ova.	Percentage of cases showing *Trichiuris* ova.	Total cases examined.	Cases showing *Ascaris* ova.	Percentage of cases showing *Ascaris* ova.	Cases showing *Trichiuris* ova.	Percentage of cases showing *Trichiuris* ova.
0– 5 days ..	318	153	48·1	112	35·2	..	..	..	..	..
6–30 ,, ..	806	345	42·8	247	31·0	34	6	23·5	5	20·6
1– 2 months ..	187	66	35·2	50	26·6	22	5	22·7	5	22·7
2– 3 ,, ..	96	29	30·2	22	22·9	19	4	21·0	4	21·0
3– 6 ,, ..	105	26	24·5	22	20·9	54	6	11·1	6	11·1
6– 9 ,, ..	78	16	20·5	12	15·3	63	5	7·9	7	11·1
9–12 ,, ..	62	10	16·1	6	9·6	41	4	9·7	3	7·3
12–15 ,, ..	37	7	18·9	8	21·6	34	2	5·9	4	11·8
15–18 ,, ..	43	4	9·3	5	11·6	26	3	11·5	3	11·5
18–21 ,, ..	26	2	7·7	4	15·3	8	2	20·6	3	30·0
21–24 ,, ..	28	2	7·1	3	10·7	12	2		3	
24–30 ,, ..	19	3	15·7	3	15·7	42	1	2·3	3	7·1
30–36 ,, ..	24	2	8·2	2	8·2	33	2	6·0	3	9·0
3– 4 years ..	16	1		2		43	1	2·3	1	2·3
4– 5 ,, ..	14	1		1		15	0		3	
5– 6 ,, ..	5	0	7·7	1	12·8	3	0		0	
6– 7 ,, ..	4	1		1		7	0	0·0	0	13·8
7– 8 ,, ..	4	0	0·0	0	0·0	4	0		1	
8–17 ,, ..	6	0	0·0	0	0·0	3	0		0	
TOTAL ..	1878	668	..	501	..	463	43	..	54	..

Though the microscopic examination for ova, other than those of hookworms, was never made exhaustive, the figures recorded in Table III show that jail life is not favourable for the propagation of either *Ascaris* or *Trichiuris* infection.

CONCLUSIONS.

1. The natural elimination of hookworms and other nematodes is a proven fact.

2. Under sanitary conditions, natural elimination alone, without the assistance of any specific treatment, appreciably lowers the intensity of the infection.

3. Natural elimination is a slow process.

4. The success of an anti-hookworm campaign will depend on the concomitant actions of sanitation and specific treatment.

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I do not think that anyone would say it gets easier to be bravely blind as the years go on. It becomes easier to do things in the dark, but the demand on the will to keep pace with normal life grows greater rather than lessens. Constant effort is extraordinarily exacting, and it is really splendid how the men meet this demand on themselves, keep up their interest in everything and maintain their notable record as workers ; some in offices, some practising as masseurs, some as poultry-farmers, and others as expert craftsmen.

There are still more than five hundred men learning to be blind in our class-rooms and workshops.

Besides those actually blinded on the battlefields, 23,000 men were discharged from the Army with seriously damaged sight, and, unfortunately, many of these are finding it necessary to come to St. Dunstan's. We are dealing, too, with a number of men whose health

does not enable them to make such quick progress as others. At the same time it becomes increasingly difficult to find for the men who are ready to start on their new life suitable homes : there is the universal shortage of houses and a great scarcity of such small properties as the poultry-farmers need. Thus the difficulty and expense of settling the men has increased while we have to face enormously multiplied costs in providing for those who are our guests, in maintaining our convalescent and holiday homes, and in carrying on the ever-increasingly important work of the After-Care of the blinded soldiers.

We have also now to meet the expense of moving our headquarters—the offices for the organisation required to look after nearly 2,000 men, and also the class-rooms and workshops. The House with its beautiful grounds so generously lent by Mr. Otto Kahn as a Hostel is no longer available : fortunately, however, we have been able to find for our new quarters another house in Regent's Park a place which for several generations has been the London Home of the Marquesses of Bute. The interior has been adapted for officers, and in the gardens the class-rooms and workshops have been re-erected. The place is near the Lake on which the blinded soldiers have taken so much pleasure in rowing, and both from the point of view of fresh air and of opportunities for unimpeded exercise the situation is ideal.

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Yours faithfully,

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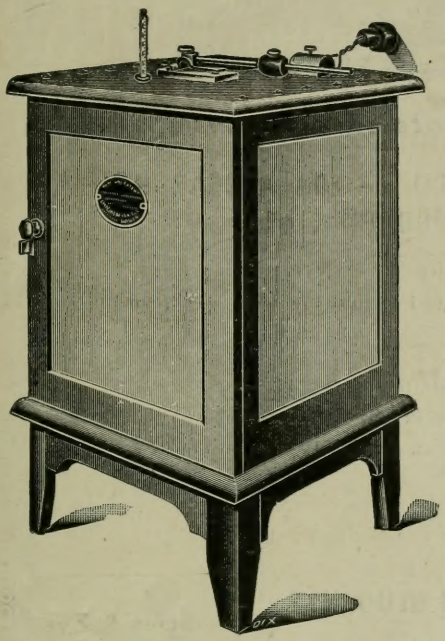

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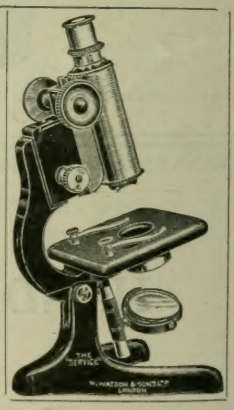

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